UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

**MEMORANDUM**

| | |
|---|---|
| **DATE:** | July 11, 2006 |
| **SUBJECT:** | Dithianon. Human Health Risk Assessment for Proposed Food Uses of the Fungicide on Imported Pome Fruit and Hops. |

| | |
|---|---|
| Petition Number: | 6E4781 |
| PC Code (Chemical Number): | 099201 |
| DP Barcode: | 235354 |
| Regulatory Citation: | 40CFR §180.XXX |
| EPA Registration Number: | No U.S. Registration |
| Trade Names: | Delan® 750 WP, Delan® 750 SC, Delan® 700 WDG, Delan® 500 SC, Ventugan 50SC |
| Chemical Class: | Quinone Fungicide |
| Regulatory Action: | Import Tolerances |

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## 1.0 Executive Summary

BASF Corp. has proposed, in PP#6E4781, the establishment of tolerances for residues of the fungicide dithianon [5,10-dihydro-5,10-dioxonaphtho(2,3-*b*)-1,4-dithiin-2,3-dicarbonitrile] in/on the following imported raw agricultural commodities:

Fruits, pome (apples and pears) . . . . . . . . 5 ppm
Hops, dried . . . . . . . . . . . . . . . . . . . . . . 100 ppm

Dithianon is a broad spectrum multi-site protectant fungicide used outside the U.S. for control of apple and pear scab, black rot, rust, and leaf spot diseases (see Table 2.1.1. for the complete list) in pome fruit, and Peronospora in hops. There are no U.S. registrations or proposed registrations of dithianon in the U.S. at this time.

Dithianon is registered for foliar uses on pome fruit in Japan, Australia, New Zealand, South Africa, the UK, Spain, Austria, France, Germany, Italy, Turkey, Israel, Argentina, Brazil, and Chile. Dithianon is registered for foliar use on hops in Germany. Codex Maximum Residue Limits (MRLs) have been established for pome fruit at 5 mg/kg and hops at 100 mg/kg; the proposed tolerances (without U.S. registration) on imported commodities are harmonized with the established Codex MRLs. There are currently no established Canadian or Mexican MRLs for dithianon.

Hazard Identification

The toxicology database is sufficient to characterize the hazards associated with dithianon, with the exception of the developmental toxicity study in rabbits, which was classified unacceptable. To account for this database gap, a 10X database uncertainty factor ($UF_{DB}$) was applied. The acute toxicity is mild via the oral route (Category III). The toxicologically significant adverse effects of dithianon are similar across species. In studies with shorter durations of exposure, including the subchronic dog and rat studies, the developmental toxicity study in rats, and the two-generation reproduction rat study, decreases in body weight, body weight gain, and/or food consumption were noted in adults. However, with continued exposure, as in the chronic and/or carcinogenicity studies in the rat, mouse, and dog, it becomes evident that the kidney is the target organ for toxicity. Signs of renal toxicity include increased absolute and/or relative kidney weights in the rat, mouse, and dog; non-neoplastic kidney lesions in mice and rats; and renal adenomas and carcinomas in female rats.

The available toxicology database does not show any indication of increased qualitative or quantitative susceptibility of the offspring. Dithianon did not cause reproductive or developmental toxicity in the two-generation reproduction study. In the developmental rat study, decreased fetal weights were observed only at a dose higher than that which produced similar maternal effects. The developmental toxicity study in rabbits was classified unacceptable/guideline. However, residual uncertainty due to this data gap is addressed through the use of a 10X database uncertainty factor ($UF_{DB}$), therefore making the degree of concern for pre- and/or postnatal toxicity low.

Dithianon is not mutagenic, as determined by the Cancer Assessment Review Committee

(CARC) on January 11, 2006. In accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the Cancer Assessment Review Committee (CARC) classified dithianon as "Suggestive Evidence of Carcinogenic Potential", based on the overall weight of the evidence. Quantitation of carcinogenic risk is not required.

<u>Endpoint Selection</u>

The acute dietary endpoint for women ages 13-49 is based on post-implantation loss due to early resorptions seen in the developmental rat study at 50 mg/kg/day (LOAEL); the NOAEL for this study is 20 mg/kg/day. Neither dose nor endpoint was selected for acute dietary exposure in the general population. The chronic dietary endpoint is based on decreased body weight gain and increased relative to body kidney weights (M&F), grossly observed kidney lesions in males (irregular surfaces, pale kidneys, cysts, and enlarged kidneys) and females (masses), and non-neoplastic lesions of the kidney in males (tubular nephrosis, renal cysts, and end-stage kidney lesions) and females (tubular nephrosis, proliferative tubules, and glomerulonephropathy) seen in the combined chronic toxicity/carcinogenicity study in rats at 30 mg/kg/day (LOAEL). The NOAEL for this study is 6 mg/kg/day. A combined uncertainty factor (UF) of 1000, including 10X factors for interspecies variability, intraspecies variability and database uncertainty, was applied to all endpoints. The 10X database uncertainty factor was applied due to the lack of an acceptable developmental study in the rabbit. The resultant acute reference dose (aRfD) and acute population adjusted dose (aPAD) for women ages 13-49 are 0.02 mg/kg. The chronic reference dose (cRfD) and chronic population adjusted dose (cPAD) are 0.006 mg/kg/day.

<u>Exposure and Risk Assessment</u>

The nature of the residue in plants is adequately understood, based upon studies of dithianon metabolism in apple, orange, and wheat. Dithianon is not highly metabolized in plants. When dithianon is metabolized, it results in a number of very polar, unidentified fragments, all in minor quantities. Goat metabolism studies indicate that dithianon is extensively metabolized in ruminants, with the most likely first step being the opening of the dithiine ring by protein thiols. Following the initial ring opening, the molecule may undergo a number of further bio-transformations yielding metabolites with chemical structures very different from the original molecule. The residue of concern (ROC) in plants and animals is dithianon *per se.* There is no reasonable expectation of finite residues in animal commodities, i.e., a 40 CFR§180.6[a][3] situation.

Acute and chronic dietary assessments were conducted using Dietary Exposure Evaluation Model (DEEM-FCID™, version 2.03), which uses food consumption data from the USDA's Continuing Surveys of Food Intake by Individuals (CSFII) from 1994 to 1998. An acute end-point was selected for only one population subgroup, females 13-49, for reproductive effects. The acute dietary assessment was based on tolerance-level residues, empirical processing factors for apple and pear juices, and 100% crop treated. The chronic analysis uses anticipated (average) residues from field trial data and assumes 100% crop treated for pome fruit and hops. Exposure to dithianon would originate from food only, because the proposed tolerances would only be established on imported commodities. With no proposed U.S. registration, there is no expectation that dithianon residues would occur in surface or ground water sources of drinking water.  The dietary analyses indicate that the expected acute and chronic dietary exposures to dithianon are below HED’s level of concern (100% of the PAD).  Females 13-49 had a risk estimate which was below HED’s level of concern, utilizing 66% of the acute population adjusted dose (aPAD) at the 95$^{th}$ percentile of exposure.  For chronic dietary (food only) exposure to dithianon, the most highly exposed subgroup is all infants (< 1 year), which utilized 55% of the cPAD.  Chronic dietary risk to all other subgroup is less than that of all infants (<1 year).  The general U.S. population utilizes 12% of the cPAD.

Dithianon is not intended for use in public, residential, or occupational settings; also, there is no expectation that exposure to dithianon residues would occur via water consumption.  Therefore, risk assessments for drinking water, residential, aggregate, and occupational exposures were not performed.

Residue analytical methods have been proposed for tolerance enforcement, and were forwarded to Biological and Economic Analysis Divisions’s Analytical Chemistry Laboratory (BEAD/ACL) for petition method validation (PMV) trials, which were successful.  A confirmatory method is needed for the proposed enforcement method for pome fruit; the GC/ECD data-collection method utilized in the field trials is adequate for confirmatory analysis of hops.  The limits of quantitation (LOQs) are 0.05 ppm for pome fruit and 0.5 ppm for hops. A study describing multiresidue testing of dithianon and its metabolites in plant and animal tissues has been submitted by the petitioner and forwarded by EPA to FDA.  Multiresidue methods are not likely to recover residues of dithianon in plant and animal tissues.

Recommendations for Tolerances and Data Needs

***860.1550 Proposed Tolerances***  HED has examined the toxicological, residue chemistry and proposed use information for dithianon and recommends in favor of the establishment of tolerances for residues of this fungicide in/on imported pome fruit and imported hops.  HED is asking for: (1) updated use information from countries where dithianon is registered (860.1500); (2) information on test substance purity for one toxicity study; (3) a confirmatory method for pome fruit; (4) additional information for some of the submitted field trials (860.1500); and, (5) additional field trials in South America; and (6) a new analytical reference standard for the EPA repository.  More details appear below in the Data Needs section.

The available crop field trial data support a tolerance of 5 ppm for Pome fruit (Crop Group 11), and 140 ppm on Dried hops cones for residues of dithianon, *per se* (as generated by the NAFTA Tolerance/MRL Harmonization spreadsheet), but HED recommends that the tolerance on hops be established at 100 ppm, in order to harmonize with the CODEX MRL (which is greater than the maximum field trial residue of 59.5 ppm).  The proposed tolerances should be revised to reflect  the correct commodity definitions as specified in Table 1.0, below.  The petitioner should submit a revised Section F for PP#6E4781 to reflect these changes.

**TABLE 1.0 Tolerance Summary for Dithianon.**

| Commodity | Proposed Tolerance (ppm) | Recommended Tolerance for parent compound only (ppm) |
|---|---|---|
| Fruit, Pome, Group 11 | 5 ppm | 5 ppm |
| Hop, dried cones | 100 ppm | 100 ppm |

**Data Needs**

HED recommends that the petitioner be requested to respond to items 1, 2, 4, and 6 as soon as possible, although this should not be necessary prior to establishment of the tolerances.

(1) Recent label use directions were submitted for 4 countries (France, Spain, Italy, and Germany). Because the submitted use pattern information for other countries with dithianon registrations is nearly 10 years old, updated information is required to confirm that the available crop field trial data reflect the maximum use patterns that are currently registered. The following additional information is required, as specified in the *NAFTA Guidance Document on Data Requirements for Tolerances on Imported Commodities* (April 2003): (A) the maximum single and annual application rates for each product, (B) the application timing as related to plant growth stage, (C) tank mixing directions (if applicable), and (D) the type of application equipment. Labels should be translated into English if necessary. If a maximum number of applications per season is not specified on a label (because that is not required by the registering country), the petitioner should provide information regarding typical application patterns.

(2) Information concerning the purity of Batch No. 162/83 of dithianon, used in MRID 44092620, Study 3 (MRID 44280407, QA/QC Draft Study Report) is requested.

(3) 860.1340 Residue Analytical Methods. A confirmatory method should be submitted for the proposed tolerance-enforcement method for pome fruit, HPLC/UV Method #HUK 460/38-01R. Alternatively, an interference study (demonstrating that none of the other pesticides registered on pome fruit interfere with the determination of dithianon) may be submitted.

(4) 860.1500 Crop Field Trials. Additional information should be submitted for the apple trials conducted in Brazil and the pear trials conducted in Australia and New Zealand (trial in Appleby, Nelson only). The petitioner should provide the application rates (lbs ai/A and/or kg ai/ha) used for these field trials.

(5) In addition, the major pome fruit importing countries have changed significantly since the submitted field trials were conducted; therefore, additional confirmatory field trials from Argentina and Chile should be conducted. The petitioner should conduct two field trials with apples in Argentina, two field trials with apples in Chile, and one field trial with pears in Argentina. <u>HED recommends requiring items 3 and 5 if and when a request is submitted for any new uses and/or new tolerances.</u>

(6) 860.1650 Submittal of Analytical Reference Standards. The available dithianon standard at the EPA National Pesticide Standards Repository is old, and should be replenished.

ENVIRONMENTAL JUSTICE CONCERNS:

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, “Federal Actions to Address Environmental Justice in Minority Populations,” http://www.eh.doe.gov/oepa/guidance/e128898.pdf).

As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrent.

**Review of Human Research:**

This risk assessment does not rely on any data from studies in which human subjects were intentionally exposed to a pesticide or other chemical.

## 2.0 Ingredient Profile

Dithianon is a broad-spectrum multi-site protectant fungicide that is used outside the U.S. for the control of scab, downy mildew, rust, and leaf spot diseases in pome fruit, stone fruit, small fruit, wine grapes, ornamentals, citrus, coffee, and vegetables; it is used in Germany for the control of downy mildew in hops. There are no registrations or proposed registrations for dithianon in the U.S. at this time. The data reviewed under this action were submitted by American Cyanamid Company, which has since been purchased by BASF Corporation.

### 2.1 Summary of Proposed Uses

The petitioner provided the Agency with a summary of the use patterns for apples, pears, and hops from approved foreign labels; product names were not included in the summary. A listing of the types of dithianon products used (and the target pests) is provided in Table 2.1.1; target pest information was provided as part of a summary of use directions based on Delan® 750 SC fungicide. A summary of the registered use patterns on hops and pome fruit is listed in Table 2.1.2. It was noted that the petitioner provided maximum application rates in terms of product applied (percentage rate, mL/hL, g/hL, cc/hL, L/1000 L, and L/ha), maximum ai applied in dilute spray (ppm), and kg ai/ha in 2000 L/ha of dilute spray volume. The maximum number of applications was provided for three products; the petitioner noted that not all countries require the label to specify the maximum number of applications per season.

The petitioner did not provide individual product names in the use pattern summary, but did list the following products (that were presumably registered to American Cyanamid):

(A) Delan® 750 WP (750 g ai/kg wettable powder),
(B) Delan® 750 SC (750 g ai/L soluble concentrate),
(C) Delan® 700 WDG (700 g ai/kg water-dispersible granule),
(D) Delan® 500 SC (500 g ai/kg soluble concentrate), and
(E) Ventugan 50SC (500 g ai/L soluble concentrate).

Based on the available data, dithianon is registered for foliar broadcast uses on pome fruit in Japan, Australia, New Zealand, South Africa, the UK, Spain, Austria, France, Germany, Italy, Turkey, Israel, Argentina, Brazil, and Chile; dithianon is registered for foliar broadcast use on hops in Germany. Product use rates for pome fruit range from 0.22 to 2.01 lb ai/A per application (0.25 to 2.25 kg ai/ha per application), with reported maximum seasonal rates ranging from 2.1 to 8.0 lb ai/A (2.4 to 9.0 kg ai/ha), based on a maximum of up to 12 seasonal applications, depending on the country of use. The reported use rate for hops is 0.67 lb ai/A per application (0.75 kg ai/ha per application) with a maximum seasonal rate of 6.7 lb ai/A (7.5 kg ai/ha) based on a maximum of 10 seasonal applications. PHIs for pome fruit range from 14 to 42 days, except in Japan, where PHIs are 90 days for apples and 60 days for pears. The PHI for hops in Germany is 21 days.

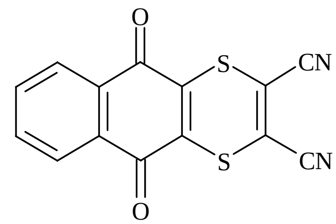
| TABLE 2.1.1 Summary of Dithianon Products Used on Pome Fruit and Hops (Outside the US). | | | |
|---|---|---|---|
| **Country** | **Formulation ai** | **Formulation Type*** | **Target Pests** |
| **Pome Fruit** | | | |
| Japan | 400 g/L | SC | Apple and pear scab (*Venturia inequalis* and *V. pirina*); summer diseases of apples including bitter rot, ripe spot, and target rot (*Gleosporium* sp.), black rot (*Physalospora obtusa*), leaf spot (*Mycosphaerella pomi*), fly speck (*Microthyriella rubi*), brown rot (*Monilinia* sp.), and apple rust (*Gymnosporangium juniperivirginianae*); and summer diseases of pear including leaf spot (*Mycosphaerella sentina*), pear rust (*Gymnosporangium sabinae*), black spot (*Alternaria kikuchiana*), and brown rot (*Monilinia* sp.). |
| New Zealand | 500 g/L | SC | |
| South Africa | 824 g/kg | WP | |
| South Africa | 500 g/L | SC | |
| Spain | 750 g/L | SC | |
| Turkey | 750 g/L | SC | |
| United Kingdom | 750 g/L | SC | |
| Australia | 750 g/L | SC | |
| Australia | 750 g/kg | WP | |
| Austria | 750 g/L | SC | |
| Argentina | 750 g/kg | WP | |
| Brazil | 750 g/kg | WP | |
| Chile | 500 g/L | SC | |
| France | 750 g/L | SC | |
| Germany | 750 g/L | SC | |
| Israel | 750 g/L | SC | |
| Italy | 750 g/L | SC | |
| Italy | 750 g/kg | WP | |
| **Hops** | | | |
| Germany | 750 g/L | SC | Peronospora. |

* SC = Soluble Concentrate, WP = Wettable Powder.

| TABLE 2.1.2 Summary of Use Directions for Dithianon. | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Country; Formulation** | **Application Type; Equipment** | **Crop** | **Maximum Application Rate (lb ai/A) [kg ai/ha]** | **Maximum Number of Applications per Season** | **Maximum Seasonal Application Rate [1] (lb ai/A) [kg ai/ha]** | **PHI (Days)** | **Use Directions and Limitations** |
| **Pome Fruit** | | | | | | | |
| Japan; 400 g/L SC | Foliar broadcast; equipment type not specified | Apple | 0.71 [0.80] | 3 | 2.14 [2.40] | 90 | Apply in a regular spray program at 7- to 14-day re-treatment intervals (RTIs), depending on disease pressure and weather. |
| | | Pear | 0.71 [0.80] | 4 | 2.86 [3.20] | 60 | |
| New Zealand; 500 g/L SC | | Apple | 0.22 [0.25] | NS [2] | NS | 42 | |
| | | Pear | 0.31 [0.35] | NS | NS | 42 | |
| South Africa; 824 g/kg WP | | Both | 1.10 [1.24] | NS | NS | 14 | |
| South Africa; 500 g/L SC | | Both | 0.40 [0.45] | NS | NS | 21 | |
| Spain; 750 g/L SC | | Both | 0.94 [1.05] | NS | NS | 21 | |
| Turkey; 750 g/L SC | | Apple | 0.67 [0.75] | NS | NS | 14 | |
| United Kingdom; 750 g/L SC | | Both | 1.00 [1.12] | 8 | 8.03 [9.00] | 28 | |
| Australia; 750 g/L SC | | Apple | 0.67 [0.75] | NS | NS | 21 | |
| | | Pear | 1.34 [1.50] | NS | NS | 21 | |
| Australia; 750 g/kg WP | | Both | 1.34 [1.50] | NS | NS | 21 | |
| Austria; 750 g/L SC | | Apple | 0.80 [0.90] | NS | NS | 35 | |
| Argentina; 750 g/kg WP | | Apple | 0.94 [1.05] | NS | NS | 21 | |
| | | Pear | 0.602 [0.675] | NS | NS | 21 | |
| Brazil; 750 g/kg WP | | Apple | 1.67 [1.88] | NS | NS | 21 | |
| Chile; 500 g/L SC | | Both | 0.80 [0.90] | NS | NS | 28 | |
| France; 750 g/L SC | | Both | 0.335 [0.375] | NS | NS | 28 | |
| Germany; 750 g/L SC | | Both | 0.67 [0.75] | 12 | 8.03 [9.00] | 21 | |
| Israel; 750 g/L SC | | Both | 1.34 [1.50] | NS | NS | NS | |
| Italy; 750 g/L SC | | Apple | 1.61 [1.80] | NS | NS | 21 | |
| | | Pear | 2.01 [2.25] | NS | NS | 21 | |
| Italy; 750 g/kg WP | | Apple | 1.61 [1.80] | NS | NS | 21 | |
| | | Pear | 2.01 [2.25] | NS | NS | 21 | |
| **Hops** | | | | | | | |
| Germany; 750 g/L SC | Foliar broadcast; equipment type not specified | Hops | 0.67 [0.75] | 10 | 6.69 [7.50] | 21 | Begin application at or before infection; repeat at 8- to 12-day intervals depending on disease pressure and weather. |

1. Maximum seasonal rates were not provided; those presented in Table 4 were calculated by the reviewer from the maximum single application rate and the number of applications per season 2. NS = Not Specified.

## 2.2 Structure and Nomenclature

| TABLE 2.2 Test Compound Nomenclature. | |
|---|---|
| Chemical structure |  |
| Common Name | Dithianon |
| Empirical Formula | $C_{14}H_4N_2O_2S_2$ |
| Company Experimental Names | CL37114, WL49890, SAG 107, CME 107, IT-931 |
| IUPAC Name | 5,10-dihydro-5,10-dioxonaphtho(2,3-*b*)-1,4-dithi-in-2,3-dicarbonitrile |
| CAS Name | 5,10-dihydro-5,10-dioxonaphtho(2,3-*b*)-1,4-dithiin-2,3-dicarbonitrile |
| CAS Registry Number | 3347-22-6 |
| Chemical Class | Quinone fungicide |
| Known Impurities of Concern | None |
| End-Use Products (EUPs) | There are no products currently registered in the U.S.; the products identified in the petition were Delan® 750 WP, Delan® 750 SC, Delan® 700 WDG, Delan® 500 SC, and Ventugan 50SC. |

## 2.3 Physical and Chemical Properties

| TABLE 2.3 Physicochemical Properties of the Technical Grade Test Compound. | | |
|---|---|---|
| **Parameter** | **Value** | **Reference** |
| Melting Point/Range (°C) | 216 | MRID 44092604 |
| pH (at 20°C) | 4.4 to 4.8 (1% wt/wt aqueous dispersion). | MRID 44092604 |
| Density (g/cm$^3$ at 20°C) | 1.58 | MRID 44092604 |
| Water Solubility (at 20°C) | Nearly insoluble (roughly 0.02 mg/100 mL). | MRID 44092604 |
| Solvent Solubility (at 20°C) | Acetone 1.76 g/100 mL<br>Dichloromethane 2.01 g/100 mL<br>Ethyl acetate 0.77 g/100 mL<br>Hexane 0.96 mg/100 mL<br>Methanol 0.08 g/100 mL<br>Toluene 1.59 g/100 mL | MRID 44092604 |
| Vapor Pressure (Pa at 25°C) | $2.71 \times 10^{-9}$ | MRID 44092604 |
| Dissociation Constant (p$K_a$) | Not available (insufficient solubility in water). | MRID 44092604 |
| Octanol/Water Partition Coefficient (Log [$K_{OW}$]) | 3.2 ± 0.3 | MRID 44092604 |
| UV/Visible Absorption Spectrum | Not provided. | |

# 3.0 Metabolism Assessment

## 3.1 Comparative Metabolic Profile

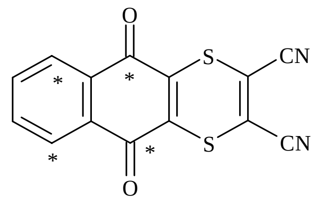
After rats were exposed to single oral doses of 10 or 50 mg/kg [$^{14}$C]-dithianon, or to repeated doses of 10 mg/kg/day unlabeled dithianon for 14 days plus 10 mg/kg [$^{14}$C]-dithianon to rats, approximately 30% of the radioactivity was excreted in the urine. Additionally, results from bile duct-cannulated rats indicated that approximately 7.2-11.6% of a single doses of either 10 or 50 mg/kg [$^{14}$C]-dithianon were recovered in the bile. Together, recovery in the urine and bile suggests that overall absorption of the test compound was approximately 40% of the administered dose. The administered radioactivity was rapidly excreted. By 120 hours following the single or repeated dosing regimens, total recoveries ranged from 96 to 98% of the administered doses, with no differences observed between doses of radioactivity or sexes. The majority of the radioactivity was recovered in the feces (64 to 72% of the administered dose) and urine (27 to 31%). Similarly, following a single dose of 10 or 50 mg/kg [$^{14}$C]-dithianon in the biliary excretion study, 43-60% of the radioactivity was recovered in the feces, 7.2-11.6% in the bile, and 24-31% in the urine. The majority of the radioactivity in the urine was excreted in the first 24 hours, while the majority of the radioactivity in the feces was excreted in the first 48 hours. Cage wash and the combined tissues and carcass accounted for no more than 0.7% of the administered dose. Tissue residue, distribution studies and whole-body autoradiography showed no buildup of radioactivity in any tissues. Radioactivity was highest in the gastrointestinal tract and kidneys. Comparison of whole blood and plasma indicated uptake of radioactivity in red blood cells. It was concluded that dithianon was degraded into a large number of mainly polar products, with none of these considered a main metabolic product. The data indicated that dithianon appeared to be broken down in the gastrointestinal tract, possibly by the resident gut flora, as only very low concentrations of the unaltered parent compound were identified in the feces. The goat metabolism study also showed extensive degradation of dithianon (see 3.2.2).

Apple, orange, and wheat metabolism studies indicate that metabolism of dithianon is similar in fruit crops and wheat. Dithianon *per se* does not appear to be highly metabolized in these plants, with a significant amount of the unchanged parent compound remaining on the plant surface. Dithianon residues accounted for 73 to 82% of the total radioactivity in fruit, and 44 to 64% of the total radioactivity in wheat forage, grain, and straw. When metabolism does occur, it results in a number of very polar, unidentifiable fragments, none of which are significant. The residue of concern in plant commodities is the parent compound only (dithianon *per se*), for both risk assessment and tolerance expression purposes.

## 3.2 Nature of the Residue in Foods

### 3.2.1. Description of Primary Crop Metabolism

American Cyanamid Company has submitted studies investigating the metabolism of [$^{14}$C]-dithianon in apples, oranges, and wheat. The studies are adequate for delineating the major dithianon residues. Metabolism of dithianon is similar in fruit crops and wheat. Dithianon does not appear to be highly metabolized in plants, with a significant amount of the unchanged parent compound remaining on the plant surface, and little to no movement from the application site.

| TABLE 3.2.1 Characteristics of Test Material Used in the Metabolism Studies. | |
|---|---|
| Chemical Structure |  |
| Radiolabel Position (*) | [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}C$]-Dithianon |

### 3.2.2 Description of Livestock Metabolism

**Ruminants**

The submitted goat metabolism study is adequate to satisfy data requirements. Although the extraction and chromatographic procedures yielded only limited qualitative and quantitative data, the analytical results for milk and tissues, in conjunction with the urinalysis results and the *in vitro* enzyme studies, confirmed that dithianon is extensively metabolized in goats. The study results are consistent with what would be expected based on the molecular structure, which is highly susceptible to attack at three reactive sites: the C=O bonds, the C-S-C bonds, and the cyano bond. The study results are also consistent with the results of the rat metabolism study, which demonstrated that dithianon was extensively degraded. HED concludes that the residue of concern in ruminant commodities is the parent compound only (dithianon *per se)*.

**Poultry**

There are no significant poultry feed items associated with the proposed uses of dithianon. Therefore, poultry metabolism data are not required to support the current petition.

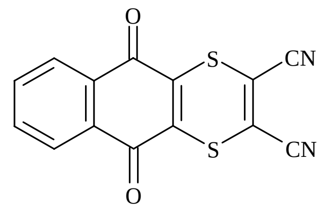
### 3.2.3 Description of Rotational Crop Metabolism

Per the NAFTA Guidance Document on Data Requirements for Tolerances on Imported Commodities (dated April 2003), rotational crop data are not required to support the proposed tolerances on imported pome fruit and hops.

## 3.3 Environmental Degradation

Since dithianon is proposed for use only on imported pome fruit and hops commodities, with neither existing nor proposed US registration, there is no expectation that dithianon residues would occur in surface or ground water sources of drinking water.

## 3.4 Tabular Summary of Metabolites and Degradates

| **TABLE 3.4 Tabular Summary of Dithianon Metabolites and Degradates.** | | |
|---|---|---|
| **Common Name**<br>***% Total Radioactive Residues*** | **Chemical Name** | **Chemical Structure** |
| Dithianon,<br>Parent Compound<br><br>***<u>Rat</u>***<br>*Essentially 0% TRR*<br>***<u>Apple Fruit [Leaves]</u>***<br>*73-82% [70-86%] TRR*<br>***<u>Orange Fruit</u>***<br>*80% TRR*<br>***<u>Wheat Grain (Husks) [Straw]</u>***<br>*44% (46%) [53%] TRR* | 5,10-dihydro-5,10-dioxonaphtho(2,3-*b*)-1,4-dithiin-2,3-dicarbonitrile |  |
| **NOTE:** In the rat, essentially all of the administered dithianon was rapidly degraded, mainly into a large number of polar products, none of which could be considered a main metabolic product. In plants, dithianon is apparently not highly metabolized; when metabolism does occur, it results in a number of very polar, unidentifiable fragments, none of which are significant. | | |
| **<u>Apple metabolism:</u>** MRID #44092624. Four or five 100µL surface applications of a 0.10% [$^{14}$C]-dithianon solution to fruit and associated foliage; fruit and foliage were harvested at PHIs of 21days after 4 treatments, and 15 days after 5 treatments.<br>**<u>Orange metabolism:</u>** MRID #44092625. Two foliar broadcast applications of a [$^{14}$C]-dithianon solution (at a total rate of 0.70 lb ai/A) to fruit and associated foliage; fruit were harvested at a PHI of 28days.<br>**<u>Wheat metabolism:</u>** MRID #44092626. Two foliar broadcast applications of a [$^{14}$C]-dithianon solution (at a total rate of 2.68 lb ai/A) to wheat plants; were harvested at a PHI of 35days.<br>**<u>Rat metabolism:</u>** MRID #44092622. Oral gavage dosing of [$^{14}$C]-dithianon, at a single low dose (10 mg/kg bw) and a single high dose (50 mg/kg bw), in mass balance/tissue distribution, bile-duct cannulated, and plasma pharmacokinetics studies; at a single low dose only (10 mg/kg bw) in a tissue distribution study; and at a single high dose (50 mg/kg bw) in a biliary metabolism study. Also, single daily oral gavage dosing of [$^{14}$C]-dithianon at the low dose (10 mg/kg bw) for 7 consecutive days in a tissue distribution study. Additionally, single daily oral gavage dosing of non-radiolabeled dithianon at the low dose (10 mg/kg bw) for 14 consecutive days, followed by a single oral gavage dose of [$^{14}$C]-dithianon (10 mg/kg bw) on the 15$^{th}$ day, in a metabolic profile study of bile, urine, feces, liver, and kidney. | | |

## 3.5 Toxicity Profile of Major Metabolites and Degradates

No toxicity data were provided for the metabolites of dithianon that were observed in apples, oranges, or wheat.

### 3.6 Summary of Residues for Tolerance Expression and Risk Assessment

| TABLE 3.6 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression. | | |
|---|---|---|
| **Crop [Matrix]** | **Residue in Risk Assessment** | **Residue in Tolerance Expression** |
| Pome Fruit (Crop Group 11) [Fruit] | Parent compound only, dithianon *per se*. | Parent compound only, dithianon *per se*. |
| Hops [Dried Cones] | | |

In the rat, essentially all of the administered dithianon was rapidly degraded, mainly into a large number of polar products, none of which could be considered a main metabolic product. In plants, dithianon is apparently not highly metabolized; when metabolism does occur, it results in a number of very polar, unidentifiable fragments, none of which are significant. Therefore, the residue of concern for both risk assessment and tolerance expression is dithianon, *per se*.

## 4.0 Hazard Characterization/Assessment

### 4.1 Hazard Characterization

#### 4.1.1 Database Summary

##### 4.1.1.1 Studies Available and Considered

Acute toxicity was evaluated using an acute oral toxicity study. Subchronic studies in the rat and dog; chronic and/or carcinogenicity studies in the rat, mouse, and dog; developmental toxicity studies in the rat and rabbit; and a two-generation reproduction study in the rat were available for hazard characterization. Consideration of multiple available mutagenicity studies and a series of metabolism studies completed the hazard characterization.

Executive summaries for all available mammalian toxicology studies on dithianon are located in the appendix.

##### 4.1.1.2 Mode of Action, Metabolism, and Toxicokinetic Data

*Mode of Action.* Dithianon is a multi-site protective fungicide that inhibits spore formation due to its strong affinity towards sulphydryl-containing compounds found in many proteins and towards thiol-containing enzymes, including glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione reductase, and malate dehydrogenase. The mode(s) of action for mammalian toxicity associated with dithianon is currently unknown.

*Metabolism and Toxicokinetic Data.* Absorption of dithianon was rapid and not affected by dose level. Radioactivity was detected in the plasma within 15 minutes, which was the first time of measurement in the available studies. In bile duct-cannulated animals exposed to a single dose of either 10 or 50 mg/kg dithianon, total recovery of the administered dose over 48 hours was 93.2-104.3%, with 7.2-11.6% of the administered dose recovered in the bile, 23.5-33.0% recovered in the urine, 1.1-6.5% recovered in the carcass, and minor amounts of radioactivity ($\leq$1.6%) found in the cage wash and the liver. Based on the amount of radioactivity recovered in the urine and bile, 31-43% of the administered dose was absorbed following a single dose.

In tissues other than the gastrointestinal tract, the highest level of radioactivity measured after exposure to 10 mg/kg dithianon was found in the kidneys. Radioactivity was also detected in the liver, plasma, and whole blood, but it was not detected in the brain or spinal cord. There were no sex-related differences in distribution.

Dithianon was rapidly metabolized to many, mostly polar, compounds. When metabolic fractions were isolated, 15 were found in urine samples, >25 fractions were found in the feces, and many were found in the kidneys and liver. Following a single oral dose, only one metabolic fraction was comprised of >5% of the radioactivity from the administered dose; that fraction was from a urine sample checked 8-24 hours after exposure and was identified as a glucuronic acid conjugate. No sex-related differences in metabolism were noted.

There was no bioaccumulation of dithianon. Recovery following repeated dosing was complete at 120 hours. At that time, following 14 daily doses of 10 mg/kg non-radioactive dithianon and a single dose of 10 mg/kg radiolabeled dithianon, 67-72% of the administered dose was found in the feces, 27-31% in the urine, 0.3-0.4% in the cage wash, and <0.2% in the carcass. A preliminary excretion study found that radioactivity was not detected in exhaled air. Excretion through the biliary route was not examined following the repeat dosing regimen; however, it was found to be a minor pathway following a single exposure, since only 7.2-11.6% of a single dose of 10 or 50 mg/kg dithianon was recovered in the bile 48 hours after exposure. The terminal half-life of dithianon was 46-57 hours. No sex- or dose-related differences on excretion were observed.

#### 4.1.1.3 Sufficiency of Studies/Data

The proposed tolerances for dithianon are for imported hops and pome fruit; thus, the acute oral toxicity study is the only study required to evaluate acute toxicity. Likewise, subchronic dermal and inhalation studies are not required at this time because residential and occupational risk assessments are not necessary to set tolerances on imported commodities. The remaining toxicology database is sufficient to characterize the hazards associated with dithianon, with the exception of the developmental toxicity study in rabbits, which was classified unacceptable. To account for this database gap, a 10X database uncertainty factor ($UF_{DB}$) was applied.

### 4.1.2 Toxicological Effects

#### 4.1.2.1 Acute Toxicity

As shown in Table 4.1, the acute toxicity of dithianon is mild via the oral route.

| TABLE 4.1 Acute Toxicity Profile for Dithianon. | | | | | |
|---|---|---|---|---|---|
| **Test Material* [% ai]** | **Guideline Number** | **Study Type** | **MRID Number** | **Results** | **Toxicity Category** |
| Technical Product | **870.1100** | Acute oral - rat | 44092605 | $LD_{50}$ (♂+♀) = 702 mg/kg (95% C.I. = 597-893 mg/kg) | III |
| Technical Product | **870.1200** | Acute dermal - rat | Not applicable for proposed use pattern (Import tolerance). | | |

| TABLE 4.1 Acute Toxicity Profile for Dithianon. | | | | | |
|---|---|---|---|---|---|
| **Test Material* [% ai]** | **Guideline Number** | **Study Type** | **MRID Number** | **Results** | **Toxicity Category** |
| Technical Product | **870.1300** | Acute inhalation - rat | Not applicable for proposed use pattern (Import tolerance). | | |
| Technical Product | **870.2400** | Acute eye irritation - rabbit | Not applicable for proposed use pattern (Import tolerance). | | |
| Technical Product | **870.2500** | Acute dermal irritation - rabbit | Not applicable for proposed use pattern (Import tolerance). | | |
| Technical Product | **870.2600** | Skin sensitization - guinea pig | Not applicable for proposed use pattern (Import tolerance). | | |

### 4.1.2.2 Subchronic, Chronic, and Other Toxicity

The toxicologically significant adverse effects of dithianon are similar across species. In studies with shorter durations of exposure, including the subchronic dog and rat studies, the developmental toxicity study in rats, and the two-generation reproduction rat study, decreases in body weights, body weight gains, and/or food consumption were noted in adults. However, with continued exposure, as in the chronic and/or carcinogenicity studies in the rat, mouse, and dog, it becomes evident that the kidney is the target organ for toxicity. Signs of renal toxicity that were observed include increased absolute and/or relative kidney weights in the rat, mouse, and dog; non-neoplastic kidney lesions in mice and rats; and renal adenomas and carcinomas in female rats.

Generally, both the body weight decreases and the kidney effects were seen at similar dose levels across species. A minor exception is the body weight changes seen in the 90-day rat and dog studies. The lowest dose that caused decreased body weights and body weight gains in dogs (*i.e.*, 13 mg/kg/day) is similar to the highest dose that failed to produce a similar response in rats (*i.e.*, 15 mg/kg/day). For comparison, the lowest dose to cause decreased body weights and body weight gains in the 90-day rat study was 87 mg/kg/day. However, this discrepancy does not mean that the dog is more sensitive than the rat; renal toxicity was observed at similar doses (*i.e.*, 35 and 30 mg/kg/day in the dog and rat, respectively) in the chronic studies.

In the species tested, males and females were equally susceptible to the effects of dithianon on body weight, body weight gain, food consumption, kidney weights, and the development of non-neoplastic renal lesions. One major difference between the sexes was seen in the carcinogenicity study in the rat, where females developed renal adenomas and carcinomas, but neoplasms were not found in the males. This sex-related difference is particularly notable because when kidney tumors develop following exposure to a given chemical, which is rare, they are generally observed in males.

Because the proposed uses for dithianon are for imported hops and pome fruits only, dermal and inhalation toxicity studies were not required for inclusion in this hazard characterization. As such, differences between routes of exposure to dithianon were not examined at this time.

The available toxicology database does not show any indication of increased qualitative or quantitative susceptibility of the offspring. Dithianon did not cause reproductive or developmental toxicity in the two-generation reproduction study. In the developmental toxicity study in rats, increased post-implantation loss due to early resorptions (significant only when total litter losses were included) was seen in conjunction with maternal toxicity ($\geq$ 50 mg/kg/day), which included decreased body weights, body weight gains, and food consumption; therefore, there is no increased quantitative susceptibility. These decreased maternal body weights were seen at $\geq$ 50 mg/kg/day, but body weights of the surviving fetuses were significantly decreased only at 100 mg/kg/day. There were no apparent treatment or dose-related external, visceral, or skeletal variations or malformations in the developmental rat study. The developmental toxicity study in rabbits was classified unacceptable/guideline due to excessive maternal toxicity that resulted in an insufficient number of litters to meet guideline requirements and excessive pre-implantation losses at all dose levels. However, residual uncertainty due to this data gap is addressed through the use of a 10X database uncertainty factor ($UF_{DB}$), so the degree of concern for pre- and/or postnatal toxicity is low.

There is no evidence of neurotoxicity in the toxicology database for dithianon.

Dithianon is not mutagenic, as determined by the Cancer Assessment Review Committee (CARC) on January 11, 2006. Dithianon produced positive results in an acceptable chromosomal aberration assay that was conducted *in vitro* using Chinese hamster lung fibroblasts (V79 cells); in contrast, a forward gene mutation assay tested in this same cell line was negative. A second forward gene mutation assay with V79 cells was also negative, but it was classified unacceptable due to inadequate cytotoxicity at the highest concentration tested. Negative responses were seen in bacteria (two acceptable reverse gene mutation assays in Salmonella), Wistar rat systems (an acceptable *in vivo* cytogenetic assay and an acceptable *in vitro* UDS assay), and NMRI mice (an unacceptable *in vivo* micronucleus assay). Although the micronucleus assay in mice was classified unacceptable because test article purity data was unavailable at the time the study was reviewed, this study was clearly negative for mutagenic effects up to a dose resulting in clinical signs of overt toxicity.

In accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the Cancer Assessment Review Committee (CARC) classified dithianon as "Suggestive Evidence of Carcinogenic Potential", based on several weight-of-evidence considerations. First, treatment-related rare kidney tumors, primarily adenomas, were seen only at the highest dose tested (600 ppm) in one sex (females) and in one species (rats). The highest dose tested was considered adequate, but not excessive, to assess the carcinogenicity of dithianon; however, significant renal toxicity occurred at this dose. Second, although the CARC concluded that there was not a sufficient or cohesive dataset at the time to fully support a mode of action, the Registrant's hypothesized non-genotoxic mode of action involving nephrotoxicity and sustained regenerative proliferation was considered to be biologically plausible. Finally, there is no mutagenicity concern for dithianon. The CARC determined that quantification of carcinogenic potential is not required.

#### 4.1.2.3 Stability of the Test Article

Questions arose regarding the stability of the test article in several of the studies. Further examination into this stability issue revealed that dithianon from Batch #15C/86 was used for all of the main toxicology studies and that dithianon, by itself, is stable. In the gavage studies, including the developmental toxicity studies in the rat and the rabbit, suspensions in 1% carboxymethylcellulose were prepared daily and found to be stable (90% nominal) for 24 hours. In the dietary studies, it was determined that an earlier analytical method used to measure the concentration of dithianon actually caused instability of the compound. When the analytical method developed later was used, dietary mixtures of dithianon as low as 40 ppm were found stable when stored under ambient conditions for one day; however, mixtures were not adequately stable when stored under ambient conditions for four days. When frozen, dietary mixtures as low as 40 ppm were stable for 8 days.

In the combined chronic toxicity/carcinogenicity study in rats, test diets were initially prepared weekly. However, early concentration analyses indicated that the low and mid dose test formulations were not stable. Therefore, from Week 7 onwards, the 20 and 160 ppm diet formulations were divided into three batches. One batch was used on the day of preparation and for the next two days. The other two batches were stored frozen until the fourth or sixth days when they were thawed at room temperature and offered to the animals for up to 2 days. Overall, the stability of the 600 ppm mixture at all storage conditions used throughout the study (= 93% nominal values) was acceptable. The stability of the 120 ppm mixture was acceptable only for the storage conditions used from Weeks 7-104 (= 89% nominal values). In contrast, even when the 20 ppm mixture was divided into three portions, none of which was stored at room temperature for more than 3 days, the stability was unacceptably low. However, because a clear LOAEL and NOAEL were seen at the high and mid dose, respectively, the poor stability of the lowest dose tested does not impact the results or acceptability of this study.

In the mouse oncogenicity study and the two-generation reproduction study, the lowest doses tested were also unstable. However, as in the two year rat study, because clear LOAELs and NOAELs were seen at the high and mid dose, respectively, the instability of the lowest dose tested does not impact the results or acceptability of these studies.

For all of the other toxicology studies, the stability of the dietary mixtures or gavage suspensions were acceptable due to better storage conditions and/or more frequent preparation. In the chronic dog study, dietary mixtures were prepared weekly and divided into 7 batches, with 6/7 of the low and mid dose preparations then stored frozen. In the remaining subchronic and developmental studies, dietary mixtures or gavage suspensions were prepared daily.

#### 4.1.3 Dose Response

Of the available toxicology studies on dithianon, adverse effects were seen only at the highest dose tested except in the developmental toxicity study in the rat. In this study, toxicity was more severe at higher doses. A dose-dependent increase in severity was seen in the decreased maternal body weights, body weight gains, and food consumption observed at 50, 70, and 100 mg/kg/day. At the highest dose tested, 100 mg/kg/day, treatment-related mortality was observed.

| TABLE 4.2 Subchronic, Chronic, and Other Toxicity Profile for Dithianon. | | | | |
|---|---|---|---|---|
| **Guideline Number** | **Study Type/ *Classification*** | **MRID Number** | **Doses** | **Results** |
| **870.3100** | 90-Day oral toxicity rodents - rat<br><br>*Acceptable/guideline* | 44092606 | 0, 30, 180, 1080 ppm<br><br>**M:** 0, 2.53, 14.64, 86.66 mg/kg/day<br>**F:** 0, 2.97, 16.32, 99.53 mg/kg/day | **NOAEL** = 14.64/16.32 mg/kg/day (M/F)<br>**LOAEL** = 86.66/99.53 mg/kg/day (M/F) based on decreased body weights and overall body weight gains in both sexes. |
| **870.3150** | 90-Day oral toxicity in nonrodents - dog<br><br>*Acceptable/guideline* | 44092607 | 0, 40, 200, 1000 ppm<br><br>**M:** 0, 0.63, 2.95, 12.58 mg/kg/day<br>**F:** 0, 0.66, 3.00, 12.61 mg/kg/day | **NOAEL** = 2.95/3.00 mg/kg/day (M/F)<br>**LOAEL** = 12.58/12.61 mg/kg/day (M/F) based on decreased body weights (F only), decreased body weight gains and food consumption (M&F), and increased alkaline phosphatase activity (M&F). |
| **870.3700** | Developmental toxicity in rodents - rat<br><br>*Acceptable/guideline* | 44092611<br>44092612 | 0, 20, 50, 70, 100 mg/kg/day<br><br>Dosing period: GD 6-15 | **Maternal**<br>**NOAEL** = 20 mg/kg/day<br>**LOAEL** = 50 mg/kg/day<br>based on decreased body weights, body weight gains, and food consumption.<br><br>At 100 mg/kg/day, 5/25 dams died between GD 13 and 17.<br>**Developmental**<br>**NOAEL** = 20 mg/kg/day<br>**LOAEL** = 50 mg/kg/day<br>based on increased incidence of total litter loss (20-42% at ≥ 50 mg/kg/day) and post-implantation loss due to early resorptions (showed decidual or placental tissues only).<br><br>At 100 mg/kg/day, weights of the surviving fetuses were decreased. |

| TABLE 4.2 Subchronic, Chronic, and Other Toxicity Profile for Dithianon. | | | | |
|---|---|---|---|---|
| **Guideline Number** | **Study Type/ *Classification*** | **MRID Number** | **Doses** | **Results** |
| **870.3700** | Developmental toxicity in nonrodents - rabbit<br><br>*Unacceptable/guideline* | 44092613<br>44092614 | 0, 10, 25, 40 mg/kg/day<br><br>Dosing period: GD 6-18, beginning prior to implantation. | The Maternal **NOAEL** and **LOAEL** could not be determined due to improper gavage techniques, which resulted in abortions and deaths.<br><br>The **developmental NOAEL and LOAEL** could not be determined due to excessive pre-implantation loss (44%, 38%, 32%, and 58% per group in the 0, 10, 25, and 40 mg/kg/day dose levels, respectively). High pre-implantation loss alters litter size, fetal weights, and other parameters, hindering the ability to assess post implantation loss. Additionally, the number of litters was insufficient to meet guideline requirements, due to high maternal mortality. |
| **870.3800** | Reproduction and fertility effects - rat<br><br>*Acceptable/guideline* | 44092615 | 0, 35, 200, 600 ppm<br><br>**M:** 0, 2.2, 12.6, 37.8 mg/kg/day<br>**F:** 0, 2.5, 14.5, 42.7 mg/kg/day | **Parental/Systemic**<br>**NOAEL** = 12.6/14.5 mg/kg/day (M/F)<br>**LOAEL** = 37.8/42.7 mg/kg/day (M/F) based on decreased body weights, body weight gains, and food consumption during pre-mating.<br><br>**Reproductive**<br>**NOAEL** = 37.8/42.7 mg/kg/day (M/F)<br>**LOAEL** = Not determined.<br><br>**Offspring**<br>**NOAEL** = 37.8/42.7 mg/kg/day (M/F)<br>**LOAEL** = Not determined. |
| **870.4100** | Chronic toxicity - rodents | See 870.4300. This study includes requirements of both 870.4100 and 870.4200. | | |
| **870.4100** | Chronic toxicity - dog<br><br>*Acceptable/guideline* | 44092608 | 0, 40, 200, 1000 ppm<br><br>**M:** 0, 1.5, 6.7, 37.1 mg/kg/day<br>**F:** 0, 1.6, 7.6, 35.0 mg/kg/day | **NOAEL** = 6.7/7.6 mg/kg/day (M/F)<br>**LOAEL** = 37.1/35.0 mg/kg/day (M/F) based on increased absolute and relative liver and kidney weights, increased alkaline phosphatase, decreased blood urea nitrogen, hepatocellular hytrophy, histiocyte pigmentation, and renal pigmentation (M&F). |

<table>
<tr><th colspan="5">TABLE 4.2 Subchronic, Chronic, and Other Toxicity Profile for Dithianon.</th></tr>
<tr><th>Guideline Number</th><th>Study Type/ Classification</th><th>MRID Number</th><th>Doses</th><th>Results</th></tr>
<tr><td>870.4200</td><td>Carcinogenicity - rat</td><td colspan="3">See 870.4300. This study includes requirements of both 870.4100 and 870.4200.</td></tr>
<tr><td>870.4200</td><td>Carcinogenicity - mouse<br><br>Acceptable/guideline</td><td>44092609<br>44092610</td><td>0, 20, 100, 500 ppm<br><br>M: ~ 0, 3, 15, 75 mg/kg/day<br>F: ~ 0, 3, 15, 75 mg/kg/day<br><br>Doses were estimated using the conversion ratio.</td><td>NOAEL = ~ 15 mg/kg/day (M&F)<br>LOAEL = ~ 75 mg/kg/day (M&F) based on increased mortality (M), increased kidney weights, (M&F), and increased incidences and severity of kidney lesions (chronic nephropathy, cortical cysts, tubular dilatation, and infarct) in both sexes.<br><br>No evidence of carcinogenicity</td></tr>
<tr><td>870.4300</td><td>Combined chronic toxicity/ carcinogenicity - rat<br><br>Acceptable/guideline</td><td>44092616<br>44092617<br>44092618</td><td>0, 20, 120, 600 ppm<br><br>M: ~ 0, 1, 6, 30 mg/kg/day<br>F: ~ 0, 1, 6, 30 mg/kg/day<br><br>Doses were estimated using the conversion ratio.</td><td>NOAEL = ~ 6 mg/kg/day (M&F)<br>LOAEL = ~ 30 mg/kg/day (M&F) based on decreased body weight gain and increased relative to body kidney weights (M&F), grossly observed kidney lesions in males (irregular surfaces, pale kidneys, cysts, and enlarged kidneys) and females (masses), and non-neoplastic lesions of the kidney in males (tubular nephrosis, renal cysts, and end-stage kidney lesions) and females (tubular nephrosis, proliferative tubles, and glomerulonephropathy).<br><br>Evidence of carcinogenicity: renal adenomas and carcinomas observed in 600 ppm females.</td></tr>
<tr><td>870.5100</td><td>Gene mutation - bacterial reverse mutation assay<br><br>Acceptable/guideline</td><td>44092619<br>44280401</td><td>0.1 - 333.3 µg/plate (-S9)<br><br>10 - 3333.3 µg/plate (+S9)</td><td>Negative.</td></tr>
<tr><td>870.5100</td><td>Gene mutation - bacterial reverse mutation assay<br><br>Acceptable/guideline</td><td>44092619<br>44280402</td><td>1 - 333.3 µg/plate (-S9)<br><br>33.3 - 3333.3 µg/plate (+S9)</td><td>Negative.</td></tr>
<tr><td>870.5300</td><td>Cytogenetics - in vitro mammalian cell gene mutation test (CHL Cells)<br><br>Unacceptable/guideline</td><td>44092619<br>44280403</td><td>0, 20, 50, 100, 200 µg/ml (-S9)<br><br>60, 150, 300, 600 µg/ml (+S9)</td><td>Negative.<br><br>This study is unacceptable due to inadequate cytotoxicity at the HDT.</td></tr>
</table>

| TABLE 4.2 Subchronic, Chronic, and Other Toxicity Profile for Dithianon. | | | | |
|---|---|---|---|---|
| **Guideline Number** | **Study Type/ *Classification*** | **MRID Number** | **Doses** | **Results** |
| **870.5300** | Cytogenetics - *in vitro* mammalian cell gene mutation test (CHO Cells)<br><br>*Acceptable/guideline* | 44092619<br>44280404 | **Trial 1**: 0.03-1.33 g/ml (-S9); 0.33-1.33 g/ml (+S9).<br>**Trial 2**: 0.33-1.00 g/ml (-S9); 0.33-1.33 g/ml (+S9).<br>**Trial 3**: 0.10-1.33 g/ml (+S9).<br>**Trial 4**: 0.03-1.00 g/ml (-S9); 0.10-1.33 g/ml (+S9). | Negative. |
| **870.5375** | Cytogenetics - *in vitro* mammalian cell chromosome aberration test<br><br>*Acceptable/guideline* | 44092620<br>44280405 | **7 hours fixation**:<br>0 or 600 ng/ml (-S9);<br>0 or 5000 ng/ml (+S9).<br><br>**18 hours fixation**:<br>0, 25, 500, 600 ng/ml (-S9); 0, 500, 1000, 5000 ng/ml (+S9).<br><br>**28 hours fixation**:<br>0 or 300 ng/ml (-S9);<br>0 or 3500 ng/ml (+S9). | Mutagenic: Evidence of structural chromosome aberrations induced over background. |
| **870.5385** | Cytogenetics - mammalian bone marrow chromosomal aberration test (rats).<br><br>*Acceptable/guideline* | 44092620<br>44280406 | 0, 22.3, 106.0, 393.5 mg/kg | Negative. |
| **870.5395** | Cytogenetics - mammalian erythrocyte micronucleus test (mice)<br><br>*Unacceptable/guideline* | 44092620<br>44280407 | 0, 1, 10, 100 mg/kg | Negative.<br><br>This study is unacceptable due to missing information on test material purity. |
| **870.5550** | Other effects - unscheduled DNA synthesis in mammalian cells in culture (rats)<br><br>*Acceptable/guideline* | 44092621 | 0, 0.1, 1.0, 5.0, 10.0, 15.0, or 20.0 g/ml for 3 hours. | Negative. |

| TABLE 4.2 Subchronic, Chronic, and Other Toxicity Profile for Dithianon. | | | | |
|---|---|---|---|---|
| **Guideline Number** | **Study Type/ *Classification*** | **MRID Number** | **Doses** | **Results** |
| **870.7485** | Metabolism and pharmacokinetics - rat<br><br>*Acceptable/guideline* | 44092622<br>44092623 | **(1)** 10 or 50 mg/kg radiolabeled, single dose by oral gavage.<br><br>**(2)** 10 mg/kg/day unlabeled, 14 days by oral gavage, PLUS 10 mg/kg radiolabeled, single dose by oral gavage.<br><br>**(3)** 10 mg/kg/day radiolabeled, 7 days by oral gavage. | **Absorption:** Rapid. Dithianon was detected in plasma within 15 min. As measured in urine and bile, 31-43% was absorbed after a single dose of 10 or 50 mg/kg (23.5-33% in urine; 7.2-11.6% in bile). Not dose-dependent.<br>**Distribution:** Besides the GI tract, highest levels in kidneys. Also detected in liver, plasma, and whole blood. Not detected in brain or spinal cord. No sex-related differences.<br>**Metabolism:** Rapidly and completely degraded to many, mostly polar, compounds. 15 fractions isolated from urine, >25 fractions from feces, many from kidneys and liver. Only 1 fraction was >5% of the radioactivity from the single administered dose; that was a glucuronic acid conjugate found in the 8-24 hr urine sample. No sex-related differences.<br>**Excretion:** No bioaccumulation. Within 120 hours after repeated exposure to 10 mg/kg/day for 14 days (unlabeled) plus 10 mg/kg (labeled) for 1 day, the radioactivity recovered was 64-72% of the administered dose in feces, 27-31% in urine, <0.7% cage wash, <0.2% in carcass, 0% in exhaled air. Biliary excretion was not measured in the repeated exposure study, although 7.2-11.6% of the radioactivity was recovered following a single dose. Therefore, % recovery for the repeated exposure study was not expressed in terms of absorbed dose. The terminal half-life was 46-57 hrs. No sex- or dose-related differences were noted. |

## 4.2 FQPA Hazard Considerations

### 4.2.1 Adequacy of the Toxicity Data Base

The toxicology database for dithianon is considered incomplete to characterize potential pre-and/or post-natal risk for infants and children. Acceptable developmental toxicity and two-generation reproduction studies in the rat were evaluated, but the available developmental toxicity study in the rabbit was classified unacceptable, for reasons discussed in 4.2.3.2. To account for this database gap, a 10X database uncertainty ($UF_{DB}$ ) was applied.

### 4.2.2 Evidence of Neurotoxicity

There is no evidence that exposure to dithianon results in neurotoxicity. No clinical signs of neurotoxicity, changes in brain weights, changes in brain gross or microscopic pathology, or any other neurotoxic effects were observed in the available acceptable studies, including the subchronic feeding studies, the chronic feeding studies, the developmental toxicity study in the rat, or the two-generation reproduction study. In the unacceptable developmental toxicity study in the rabbit, there was also no evidence of neurotoxicity, although excessive maternal mortality caused by gavage errors may confound interpretation of this study. Finally, in a metabolism study, radiolabeled dithianon was not detected in the brain or spinal cord.

### 4.2.3 Developmental Toxicity Studies

#### 4.2.3.1 Developmental Toxicity Study in Rats

Increased quantitative or qualitative susceptibility was not seen in the developmental toxicity study in rats. (See section A-2.3 of the appendix for the executive summary of MRID 44092611). Decreases in maternal body weights, body weight gains, and food consumption were seen at ≥50 mg/kg/day; however, in the fetus, decreases in body weights were only observed at 100 mg/kg/day, which was the highest dose tested. Increased post-implantation loss due to early resorptions was seen at ≥50 mg/kg/day when dams with complete litter losses were included, but this effect was only significant at 100 mg/kg/day when dams with total litter losses were excluded from the analysis. There were no apparent treatment or dose-related external, visceral, or skeletal variations or malformations.

#### 4.2.3.2 Developmental Toxicity Study in Rabbits

The developmental toxicity study in rabbits was classified unacceptable/guideline due to excessive maternal toxicity, including mortality and abortions, caused by improper gavage methodology that resulted in an insufficient number of litters to meet guideline requirements. Additionally, excessive pre-implantation loss was noted at all dose levels, likely because dosing began on GD6, prior to the day of implantation. High pre-implantation loss alters litter size, fetal weights, and other parameters, confounding interpretation of offspring toxicity in survivors. Therefore, maternal and offspring NOAELs and LOAELs were not determined for this study. (See section A-2.3 of the appendix for the executive summary of MRID 44092613).

### 4.2.4 Reproductive Toxicity Study

Increased quantitative or qualitative susceptibility was not seen in the two-generation reproduction study in rats. (See section A-2.4 of the appendix for the executive summary of MRID 44092615). At the highest dose tested (37.8/42.7 mg/kg/day in M/F), decreased body weights, body weight gains, and food consumption were seen in adult males and females during the pre-mating period. In contrast, no reproductive or offspring toxicity was observed at any of the doses tested.

#### 4.2.5 Additional Information from Literature Sources

Dithianon is currently registered in multiple countries outside the United States for use on pome fruits and hops. As such, a monograph on the toxicological evaluation of dithianon published in 1992 by the Joint Meeting on Pesticide Residues (JMPR) is available. The JMPR found that, based on the available genotoxicity data, dithianon is not mutagenic. The meeting concluded that dithianon induces kidney tumors in rats at 600 ppm; JMPR hypothesizes that tumor induction is secondary to renal toxicity in rats. However, after consideration of the toxicology database, the meeting determined that "dithianon did not pose a carcinogenic hazard for humans."

The EU monograph is expected in October 2006.

#### 4.2.6 Pre-and/or Post-Natal Toxicity

##### 4.2.6.1 Determination of Susceptibility

There is no indication of increased quantitative or qualitative susceptibility of the offspring in the developmental and two-generation reproduction studies. In the developmental toxicity study in rats, reductions in maternal body weights, body weight gains, and food consumption were seen at 50 mg/kg/day, but a higher dose (100 mg/kg/day) was required to produce a reduction in fetal body weights. The significant increase in post-implantation loss due to early resorptions that occurred at 50 mg/kg/day, including dams that experienced total litter loss, is not evidence of increased qualitative susceptibility; instead, it is likely due to maternal toxicity. The developmental toxicity study in rabbits was classified unacceptable (see section 4.2.3.2). Because maternal and offspring toxicity were not characterized for this study, a determination of susceptibility was not made. In the two-generation reproduction study, decreased body weights, body weight gains, and food consumption were observed in the parents, but no adverse effects were seen in the offspring up to the highest dose tested.

##### 4.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Post-Natal Susceptibility

The toxicology database for dithianon shows no evidence of increased qualitative or quantitative susceptibility in the offspring. However, it is incomplete with respect to pre- and post-natal toxicity since an acceptable developmental toxicity study in the rabbit is not available. Because this residual uncertainty is addressed through the use of a 10X database uncertainty factor ($UF_{DB}$), the degree of concern for pre- and/or postnatal toxicity is low.

### 4.3 Recommendation for a Developmental Neurotoxicity Study

At this time, a developmental neurotoxicity study on dithianon is not required.

### 4.3.1 Evidence that Supports Requiring a Developmental Neurotoxicity Study

There is no evidence to support the requirement for a developmental neurotoxicity study on dithianon.

### 4.3.2 Evidence that Supports not Requiring a Developmental Neurotoxicity Study

There was no evidence of neurotoxicity in any of the acceptable studies available in the toxicology database, including the subchronic feeding studies, the chronic feeding studies, the developmental toxicity study in rats, and the two-generation reproduction study. In the unacceptable developmental toxicity study in the rabbit, there was also no evidence of neurotoxicity, although excessive maternal mortality caused by gavage errors may confound interpretation of this study. In a metabolism study (MRID 44092622), radiolabeled dithianon was not detected in the brain or spinal cord. Because there is no evidence of neurotoxicity in the available toxicology studies and there is no evidence of increased qualitative or quantitative susceptibility in the offspring, a developmental neurotoxicity study is not required.

#### 4.3.2.1 Rationale for the $UF_{DB}$

The database uncertainty factor is 10X, based on the lack of an acceptable developmental toxicity study in rabbits.

## 4.4 Hazard Identification and Toxicity Endpoint Selection

### 4.4.1 Acute Reference Dose (aRfD) - Females (13 to 49 Years of Age)

An appropriate endpoint attributable to a single exposure was identified from the developmental toxicity rat study. The acute RfD (aRfD) and aPAD of 0.20 mg/kg/day for females 13-49 are derived from a NOAEL of 20 mg/kg/day. The LOAEL was 50 mg/kg/day, based on post-implantation loss because of early resorptions. There was no appropriate endpoint attributable to a single exposure identified for the general population or for any other population subgroup.

***Study Selected:*** Developmental toxicity study in rats
***MRID Number:*** 44092611.
***Executive Summary:*** See Section A-2.3 of the appendix.
***Dose and Endpoint for Establishing aRfD:*** 20 mg/kg/day (NOAEL), based on post-implantation loss due to early resorptions seen at 50 mg/kg/day (LOAEL).
***Uncertainty Factor(s):*** 1000X (10X for interspecies variability, 10X for intraspecies variability, 10X for database uncertainty)
***Comments about Study/Endpoint/Uncertainty Factor:***
Post-implantation loss resulting from early resorptions can be attributed to a single oral dose and is an appropriate endpoint for the population of concern.

**Acute RfD (Females 13-49 Years of Age** $= \dfrac{\textbf{20 mg/kg/day (NOAEL)}}{\textbf{1000 (UF)}} =$ **0.02 mg/kg/day**

#### 4.4.2 Acute Reference Dose (aRfD) - General Population

No appropriate dose and endpoint could be identified to set a reference dose for acute dietary exposure in the general population, including infants and children. No neurotoxic effects, maternal toxicity following 1 or 2 doses in the developmental studies, or other effects following a single oral dose that appeared to affect this population group were seen.

#### 4.4.3 Chronic Reference Dose (cRfD)

***Study Selected:*** Combined chronic toxicity/carcinogenicity study in rats.
***MRID Number:*** 44092616.
***Executive Summary:*** See Section A-2.5 of the appendix.
***Dose and Endpoint for Establishing cRfD:*** 6 mg/kg/day (NOAEL), based on decreased body weight gain and increased relative to body kidney weights (M&F), grossly observed kidney lesions in males (irregular surfaces, pale kidneys, cysts, and enlarged kidneys) and females (masses), and non-neoplastic lesions of the kidneys in males (tubular nephrosis, renal cysts, and end-stage kidney lesions) and females (tubular nephrosis, proliferative tubules, and glomerulonephropathy) seen at 30 mg/kg/day (LOAEL).
***Uncertainty Factor(s):*** 1000X (10X for interspecies variability, 10X for intraspecies variability, 10X for database uncertainty)
***Comments about Study/Endpoint/Uncertainty Factor:*** The chronic duration and dietary route of exposure in the selected study, as well as the types of adverse effects observed, are appropriate to set a chronic reference dose.

The chronic dog study (MRID 44092608) was considered a co-critical study because the NOAEL, LOAEL, and target organ toxicity were similar to what was seen in the combined chronic/carcinogenicity rat study. The executive summary for the chronic dog study is found in Section A-2.5 of the appendix. In this study, the LOAEL is 37.1/35 mg/kg/day [M/F]), based on increased absolute and relative liver and kidney weights, increased alkaline phosphatase, decreased blood urea nitrogen, hepatocellular hytrophy, histiocyte pigmentation, and renal pigmentation (M&F). The NOAEL for this study, 6.7/7.6 mg/kg/day [M/F], is well established and could have detected changes in body weights in the 90-day dog study. Therefore, the lower NOAEL found in the 90-day dog study, 2.95/3.00 mg/kg/day [M/F], is an artifact of dose selection.

The chronic reference dose is based on the NOAEL (6 mg/kg/day) from the combined chronic/carcinogenicity rat study, which is well established and addresses concerns (*i.e.*, is protective of the effects) seen in the dog.

$$\textbf{Chronic RfD} = \frac{\textbf{6 mg/kg/day (NOAEL)}}{\textbf{1000 (UF)}} = \textbf{0.006 mg/kg/day}$$

#### 4.4.4 Incidental Oral Exposure (Short and Intermediate Term)

Because the proposed uses for dithianon include only imported pome fruit and hops, the sole anticipated exposure route for the US population is via the diet (food only). Therefore, residential and occupational exposure risk assessments for the incidental oral, dermal, and inhalation exposure routes are not required at this time.

#### 4.4.5 Dermal Absorption

See Section 4.4.4.

#### 4.4.6 Dermal Exposure (Short, Intermediate and Long Term)

See Section 4.4.4.

#### 4.4.7 Inhalation Exposure (Short, Intermediate and Long Term)

See Section 4.4.4.

#### 4.4.8 Margins of Exposure

See Section 4.4.4.

#### 4.4.9 Recommendation for Aggregate Exposure Risk Assessments

An aggregate assessment is not required at this time. See Section 7.0.

#### 4.4.10 Classification of Carcinogenic Potential

In accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the Cancer Assessment Review Committee (CARC) classified dithianon as "Suggestive Evidence of Carcinogenic Potential", based on several weight-of-evidence considerations. First, treatment-related rare kidney tumors, primarily adenomas, were seen only at the highest dose tested (600 ppm) in one sex (females) and in one species (rats). The highest dose tested was considered adequate, but not excessive, to assess the carcinogenicity of dithianon; however, significant renal toxicity occurred at this dose. Second, although the CARC concluded that there was not a sufficient or cohesive dataset at the time to fully support a mode of action, the Registrant's hypothesized non-genotoxic mode of action involving nephrotoxicity and sustained regenerative proliferation was considered to be biologically plausible. Finally, there is no mutagenicity concern for dithianon. The CARC determined that quantification of carcinogenic potential is not required.

### 4.5 FQPA Safety Factor

Based on the hazard and exposure data, the dithianon risk assessment team has recommended that the FQPA SF be reduced to 1X because there are no/low concerns with regard to pre- and/or postnatal toxicity, and residual uncertainty has been addressed. This recommendation is based on the following:

(1) residual uncertainty concerning the lack of an acceptable developmental toxicity study in rabbits has been addressed through the use of a 10X database uncertainty factor ($UF_{DB}$);

(2) there is no indication of increased quantitative or qualitative susceptibility of rats to *in utero* and/or postnatal exposure to dithianon;

(3) the dietary food exposure assessment utilizes average residues from crop field trials and 100% crop treated information for all commodities; by using these screening-level assessments, acute and chronic exposures/risks will not be underestimated; and
(4) there are no existing or proposed residential uses for dithianon at this time.

**Table 4.3 Summary of Toxicological Doses and Endpoints for Dithianon to be Used in Human Health Risk Assessments.**

| Exposure Scenario | Dose Used in Risk Assessment and UF | FQPA SF* and Level of Concern for Risk Assessment | Study and Toxicological Effects |
|---|---|---|---|
| Acute Dietary (females 13-49 years of age) | NOAEL = 20 mg/kg/day<br>UF = 1000 [a]<br><br>**Acute RfD** = 0.02 mg/kg/day | FQPA SF = 1<br>**aPAD** = acute RfD / FQPA SF<br><br>= 0.02 mg/kg/day | **Developmental toxicity study in rats**<br>LOAEL = 50 mg/kg/day<br>based on post-implantation loss due to early resorptions |
| Acute Dietary (general population, including infants and children) | None | None | **Not selected**<br>No appropriate dose and endpoint could be identified for these population groups. |
| Chronic Dietary (all populations) | NOAEL = 6 mg/kg/day<br>UF = 1000 [a]<br><br>**Chronic RfD** = 0.006 mg/kg/day | FQPA SF = 1<br>**cPAD** = chronic RfD / FQPA SF<br><br>= 0.006 mg/kg/day | **Combined chronic toxicity/oncogenicity study in rats**<br>LOAEL = 30 mg/kg/day<br>based on decreased body weight gains and increased relative to body kidney weights (M&F), grossly observed kidney lesions in males (irregular surfaces, pale kidneys, cysts, and enlarged kidneys) and females (masses), and non-neoplastic lesions of the kidney in males (tubular nephrosis, renal cysts, and end-stage kidney lesions) and females (tubular nephrosis, proliferative tubules, and glomerulonephropathy). |
| Incidental Oral (all durations) | None | None | **Not selected**<br>Tolerance on imported commodities - no proposed uses would result in residential exposure in the US. |
| Dermal (all durations) | None | None | **Not selected**<br>Tolerance on imported commodities - no proposed uses would result in residential or occupational exposure in the US. |
| Inhalation (all durations) | None | None | **Not selected**<br>Tolerance on imported commodities - no proposed uses would result in residential or occupational exposure in the US. |
| Cancer (oral, dermal, inhalation) | **Classification:** Classification is "Suggestive Evidence of Carcinogenic Potential". The risk assessment for chronic effects is considered protective of any cancer effect. | | |

UF = uncertainty factor, FQPA SF = FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL =

lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, N/A = Not Applicable. * Refer to Section 4.5. a Additional 10x database uncertainty factor for lack of an acceptable developmental rabbit study.

### 4.6 Endocrine Disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

In the available toxicity studies on dithianon, no estrogen-, androgen-, and/or thyroid-mediated toxicity was observed.

When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, dithianon may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

## 5.0 Public Health Data

Dithianon is a new active ingredient in the U.S., and, as such, no public health data are currently available.

## 6.0 Exposure Characterization/Assessment

### 6.1 Dietary Exposure/Risk Pathway

#### 6.1.1 Residue Profile

The submitted crop field trials for pome fruit and hops are adequate to support tolerances on these imported commodities. Additional, confirmatory field trial data are being requested. The residue analytical methods used are considered adequate for enforcement and data collection.

**Pome Fruit (Crop Group 11)** The submitted pome fruit field trial data are adequate to satisfy data requirements. Additional information for the apple trials conducted in Brazil, and for the pear trials conducted in Australia and New Zealand (trial in Appleby, Nelson only) should be provided on the application rates used (expressed in terms of lbs ai/A or kg ai/ha).

**<u>The field trial data submitted by the petitioner were generated more than 10 years ago, and at that time, the chosen countries may have adequately represented the major countries</u>**

**<u>producing pome fruit imported by the US. Because the major countries have changed significantly since the field trials were conducted, HED concludes that additional confirmatory field trials from Argentina and Chile should be submitted. The petitioner should conduct two field trials with apples in Argentina, two field trials with apples in Chile, and one field trial with pears in Argentina.</u>**

Apple and pear samples from the Australia, France, New Zealand, and Brazil field trials were analyzed for residues of dithianon using an HPLC/UV method with a validated LOQ of 0.05 ppm in apples and pears. Apple samples from the German field trials were analyzed using a HPLC/UV method with a validated LOQ of 0.02 ppm in apples. These methods are adequate for data collection, based on acceptable concurrent method recovery data.

The available storage stability data indicate that residues of dithianon are reasonably stable in apples for up to 24 months of frozen storage. Dithianon residues in pears declined to about 70% of the original levels after 6 months of storage, then remained at that level for up to 24 months (total) of frozen storage.

The submitted field trial data for apples and pears generally reflect exaggerated application rates and/or shorter PHIs than the requested uses from the countries in which the trials were conducted. However, the application rates and PHIs were within the overall range of application rates and PHIs for all countries with dithianon uses on pome fruit, and can be used to establish a tolerance on imported pome fruit. For the apple trials in Brazil and the pear trials in Australia and New Zealand (one trial only), insufficient information was provided to allow determination of the application rate; this information should be submitted. The maximum dithianon residue observed in pome fruit was 3.67 ppm. The results of the residue decline trials indicate that residues of dithianon generally decline in pome fruit with increasing sampling intervals.

The submitted apple processing data are adequate to satisfy data requirements. The processing data indicate that residues of dithianon concentrate in apple wet pomace (3.2X).

**Hops.** The submitted hops field trial residue data are adequate to satisfy data requirements. The petitioner submitted 4 field trial studies from Germany, the only country in which dithianon is used on hops. Green and dried hops cones were analyzed using a GC/ECD Method with a reported LOQ of 0.05 ppm. The methods are adequate for data collection, based on acceptable concurrent method recovery data. Adequate storage stability data are available to support the storage conditions and durations of hop samples from the field trials.

Only the 1995 trials included the 21-day PHI reflected in the registered use pattern for hop. At those trials, the maximum residue of dithianon observed was 59.5 ppm in dried hop cones (harvested at 21 DALA). Based on the results of the four 1995 decline trials, residues of dithianon generally declined in dried hop cones with subsequent sampling intervals.

***<u>860.1340 Residue Analytical Method (Plant Commodities)</u>***

An HPLC/UV method, Method No. HUK 460/38-01R, for the enforcement of a tolerance for residues of dithianon in pome fruit, and an HPLC/ECD method, Method No. M 2600, for the enforcement of a tolerance for residues of dithianon in dried hop cones were submitted. Adequate independent laboratory validation data have been submitted for the method using samples of pear.  Based on the results of plant metabolism studies, it is concluded that radiovalidation data are not required for the method.  The validated LOQ is 0.05 ppm for pome fruit; no LOD was reported.  A confirmatory method has not been proposed.

Hop analytical method:  The proposed HPLC/ECD enforcement method is entitled "CL 37,114 (dithianon):  HPLC Methods for the Determination and Confirmation of CL 37,114 Residues in Dried Hops Cones."   The validated LOQ is 0.5 ppm for dried hop cones; no LOD was reported. The GC/ECD data-collection method utilized on the hops field trial samples can be used for confirmatory analysis.

Adequate independent laboratory validation data have been submitted for the method using samples of dried hops cones; radiovalidation data are not required in consideration of the plant metabolism study results.  No method validation data were provided in support of the proposed enforcement method; however, the ILV data included in the submission reflect fortification levels that are sufficiently representative of the expected residue levels for dried hops cones.

Residue analytical methods have been proposed for tolerance enforcement, and were forwarded to Biological and Economic Analysis Divisions's Analytical Chemistry Laboratory (BEAD/ACL) for petition method validation (PMV) trials, which were successful

#### 6.1.2 Acute and Chronic Dietary Exposure and Risk

The dietary exposure assessment, entitled *Dithianon Acute and Chronic Dietary Exposure Assessments for the Tolerances (Without US Registration) on Imported Pome Fruit (Crop Group 11) and Imported Hops*, was conducted by William T. Drew, 7/7/2006, D324044.

Acute and  chronic dietary exposure risk assessment were conducted for the new active ingredient dithianon using the Dietary Exposure Evaluation Model (DEEM-FCID, Version 2.03), which uses food consumption data from the USDA's *Continuing Surveys of Food Intakes by Individuals* (CSFII) from 1994 to 1996, and 1998.  The analysis was performed to support the requests for tolerances on imported pome fruit (Crop Group 11) and imported hops.

***<u>Acute Dietary Exposure:</u>***   The acute dietary exposure analysis was based on the following assumptions:
(1) tolerance-level residues of dithianon in/on pome fruit (5 ppm) and hops (100 ppm) raw agricultural commodities (RACs);
(2) the empirical processing factor obtained from the apple processing study data for apple and pear juices;
(3) default DEEM™ 7.81 processing factors for the remaining pome fruit processed commodites; and,
(4) 100% crop treated (CT).

An apple processing study was submitted which indicated that dithianon residues do not concentrate in apple juice; the average processing factor was 0.2X.  An acute endpoint was selected for only one population subgroup, females 13-49.  This subgroup had a risk estimate which was below HED's level of concern, utilizing 66% of the acute population adjusted dose (aPAD) at the 95$^{th}$ percentile of exposure.

***Chronic Dietary Exposure:*** A conservative chronic dietary exposure analysis was performed for dithianon. The analysis is based on anticipated (average) residues and the assumption that 100% of the crop will be treated. The risk estimates for all population subgroups are below HED's level of concern. The risk estimate for the general US population is 12% of the cPAD. The most highly exposed representative population subgroup is all infants (<1 year), which utilizes 55% of the cPAD.

| TABLE 6.1 Summary of Dietary Exposure and Risk for Dithianon. | | | | | | |
|---|---|---|---|---|---|---|
| **Population Subgroup** | **Acute Dietary (95th Percentile)*** | | | **Chronic Dietary*** | | |
| | **aPAD (mg/kg)** | **Exposure (mg/kg/day)** | **% aPAD** | **cPAD (mg/kg/day)** | **Exposure (mg/kg/day)** | **% cPAD** |
| General US Population | Not applicable. | | | 0.006 | 0.000738 | 12 |
| **All Infants <1 year** | | | | **0.006** | **0.003268** | **55** |
| Children 1-2 years | | | | 0.006 | 0.002773 | 46 |
| Children 3-5 years | | | | 0.006 | 0.001995 | 33 |
| Children 6-12 years | | | | 0.006 | 0.000903 | 15 |
| Youths 13-19 years | | | | 0.006 | 0.000313 | 5 |
| Adults 20-49 years | | | | 0.006 | 0.000583 | 10 |
| Adults 50+ years | | | | 0.006 | 0.000483 | 8 |
| Females 13-49 years | 0.02 | 0.013119 | 66 | 0.006 | 0.000369 | 6 |

* Values for the population with the highest risk are **bolded**.

### 6.2 Water Exposure/Risk Pathway

Since dithianon is proposed for use only on imported pome fruit and imported hops commodities, the sole anticipated exposure route for the US population is via dietary (food) exposure. With no proposed US registration, there is no expectation that dithianon residues would occur in surface or ground water sources of drinking water.

### 6.3 Residential (Non-Occupational) Exposure/Risk Pathway

See Section 6.2. Therefore, residential exposure is not expected and no residential risk assessment was performed

## 7.0 Aggregate Risk Assessments and Risk Characterization

See Section 6.2. Therefore, aggregate exposure is not expected and no aggregate risk assessment was performed.

## 8.0 Cumulative Risk Characterization/Assessment

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to dithianon and any other substances, and dithianon does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that dithianon has a common mechanism of toxicity with other substances.

For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs (OPP) concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

## 9.0 Occupational Exposure/Risk Pathway

Since dithianon is proposed for use only on imported commodities, the sole anticipated exposure route for the US population is via dietary (food) exposure. With no proposed U.S. registration, there is no expectation that exposure to dithianon residues would occur via occupational use. Therefore, no occupational risk assessment was performed.

## 10.0 Data Needs and Label Requirements

### 10.1 Toxicology

Information concerning the purity of Batch No. 162/83 of dithianon, used in MRID 44092620, Study 3 (MRID 44280407, QA/QC Draft Study Report) is requested.

### 10.2 Residue Chemistry

HED has examined the residue chemistry database for dithianon. The registrant should submit additional information pertaining to the directions for use and the crop field trials, and should submit a revised Section F and a new analytical reference standard.

**Additional residue chemistry data are needed. These include information on apple trials already conducted in Brazil and pear trials already conducted in Australia and New Zealand (trial in Appleby, Nelson only). In addition, because the major pome fruit exporting countries have changed significantly since the submitted field trials were conducted, additional confirmatory field trials from Argentina and Chile should be conducted. The petitioner should conduct two field trials with apples in Argentina, two field trials with apples in Chile, and one field trial with pears in Argentina.**

***<u>860.1200 Directions for Use</u>***
**(1)** Because the submitted use pattern information is nearly 10 years old, updated information is required to confirm that the available crop field trial data reflect the maximum use patterns that are currently registered (recent information was only submitted for France, Spain, Italy and Germany). The following additional information is required, as specified in the *NAFTA Guidance Document on Data Requirements for Tolerances on Imported Commodities* (April 2003):

(A) the maximum single and annual application rates for each product,
(B) the application timing as related to plant growth stage,
(C) tank mixing directions (if applicable), and
(D) the type of application equipment.

Labels should be translated into English if necessary.  If a maximum number of applications per season is not specified on a label (because that is not required by the registering country), the petitioner should provide information regarding typical application patterns.

***860.1340 Residue Analytical Methods***
**(2)** A confirmatory method should be submitted for the proposed tolerance-enforcement method for pome fruit, HPLC/UV Method #HUK 460/38-01R.  Alternatively, an interference study (demonstrating that none of the other pesticides registered on pome fruit interfere with the determination of dithianon) may be submitted.

***860.1500 Crop Field Trials***
**(3a)** Additional information should be submitted for the apple trials conducted in Brazil and the pear trials conducted in Australia and New Zealand (trials in Appleby, Nelson only).  The petitioner should provide the application rates calculated in terms of lb ai/A and/or kg ai/ha.
**(3b)** Because the major pome fruit exporting countries have changed significantly since the submitted field trials were conducted, additional confirmatory field trials from Argentina and Chile should be conducted.  The petitioner should conduct two field trials with apples in Argentina, two field trials with apples in Chile, and one field trial with pears in Argentina.

HED is not requiring these data in conjunction with the current petition, but will require the additional pome fruit field trials and a confirmatory method (item 2) if and when a request is submitted for any new uses and/or new tolerances

***860.1500 Proposed Tolerances***
**(4)** HED has determined that the terminal residue of concern in plant and ruminant commodities is dithianon *per se*.  The tolerance expression proposed in this petition is appropriate.

Codex MRLs have been established for pome fruit at 5 mg/kg, and hops at 100 mg/kg; the proposed tolerances (without US registration) on imported commodities are harmonized with established MRLs.  Codex MRLs have also been established for cherries, grapes, mandarins, and pomelos.  There are currently no established Canadian or Mexican MRLs for dithianon.  An International Residue Limit Status sheet is attached to this review.

The available crop field trial data will support the establishment of  tolerances (without US registration) on imported commodities for residues of dithianon *per se* in/on hops and pome fruit.  It is noted that there are currently no dithianon registrations in the U.S.  The proposed tolerances (without US registration) should be revised to reflect the correct commodity definitions, “Fruit, Pome, Group 11" and “Hop, Dried Cones” as specified in Table 10.2, below.  The petitioner should submit a revised Section F for PP#6E4781 to reflect these changes.

***860.1650 Submittal of Analytical Reference Standards***
**(5)** The available dithianon standard at the EPA National Pesticide Standards Repository is old and should be replenished.

| TABLE 10.2 Tolerance Summary for Dithianon. | | | |
|---|---|---|---|
| **Commodity** | **Proposed Tolerance (ppm)** | **Recommended Tolerance (ppm)** | **Comments/ *Correct Commodity Definition*** |
| Fruits, Pome (Apple and Pear) | 5 | 5 | *Fruit, Pome, Group 11* |
| Hops, Dried | 100 | 100 | *Hop, Dried Cones* |

**References:**


(1) *Dithianon Chronic Dietary Exposure Assessments for Tolerances (Without US Registration) on Imported Pome Fruit (Crop Group 11) and Imported Hops*, D324044, William T. Drew; 7/7/2005.

(2) *Dithianon. Tolerance Petition Requesting Food Use of the Fungicide Dithianon on Imported Pome Fruit and Imported Hops (Without US Registration). Summary of Analytical Chemistry and Residue Data.* D312241; William T. Drew, 7/11/2005.

(3) *Evaluation of the Carcinogenic Potential of Dithianon,* TXR no. 0054117; 2/23/2006.

(4) Dithianon Toxicology Data Evaluation Records. D310309, Kelly Schumacher, 6 /22/2006.

## Appendices

## A-1.0 TOXICOLOGY DATA REQUIREMENTS

The requirements (40 CFR 158.340) for food use (tolerances on imported commodities only) of dithianon are in Appendix Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

**APPENDIX TABLE 1 Toxicology Data Requirements.**

| Test | Technical | |
|---|---|---|
| | Required | Satisfied |
| 870.1100 Acute Oral Toxicity | yes | yes |
| 870.1200 Acute Dermal Toxicity | no | N/A |
| 870.1300 Acute Inhalation Toxicity | no | N/A |
| 870.2400 Primary Eye Irritation | no | N/A |
| 870.2500 Primary Dermal Irritation | no | N/A |
| 870.2600 Dermal Sensitization | no | N/A |
| 870.3100 Oral Subchronic (rodent) | yes | yes |
| 870.3150 Oral Subchronic (nonrodent) | yes | yes |
| 870.3200 21-Day Dermal | no | N/A |
| 870.3250 90-Day Dermal | no | N/A |
| 870.3465 90-Day Inhalation | no | N/A |
| 870.3700a Developmental Toxicity (rodent) | yes | yes |
| 870.3700b Developmental Toxicity (nonrodent) | yes | no |
| 870.3800 Reproduction | yes | yes |
| 870.4100a Chronic Toxicity (rodent) | yes | yes |
| 870.4100b Chronic Toxicity (nonrodent) | yes | yes |
| 870.4200a Oncogenicity (rat) | yes | yes |
| 870.4200b Oncogenicity (mouse) | yes | yes |
| 870.4300 Chronic/Oncogenicity | yes | yes |
| 870.5100 Mutagenicity—Gene Mutation - bacterial | yes | yes |
| 870.5300 Mutagenicity—Gene Mutation - mammalian | yes | yes |
| 870.5375 Mutagenicity—Structural Chromosomal Aberrations | yes | yes |
| 870.5xxx Mutagenicity—Other Genotoxic Effects | yes | yes |
| 870.6100a Acute Delayed Neurotox. (hen) | no | N/A |
| 870.6100b 90-Day Neurotoxicity (hen) | no | N/A |
| 870.6200a Acute Neurotox. Screening Battery (rat) | no | N/A |
| 870.6200b 90 Day Neuro. Screening Battery (rat) | no | N/A |
| 870.6300 Develop. Neuro | no | N/A |
| 870.7485 General Metabolism | yes | yes |
| 870.7600 Dermal Penetration | no | N/A |

## A-2.0 TOXICOLOGY STUDIES

Note: The executive summaries from the toxicology data evaluation records have been slightly modified for purposes of this risk assessment; the content has not been changed.

### A-2.1 Acute Studies

**Acute Oral Toxicity (870.1100)**

In an acute oral toxicity study (MRID 44092605), 5/sex/group Wistar rats (Age: 9-12 weeks; Weight: 193-252 g males, 172-209 g females; Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland) were given an oral dose of Dithianon (purity: 92 +- 0.6%; Batch #: 15C/86; brown powder) by oral gavage at a dose of 100, 400, 600, 1,000, or 5,000 mg/kg. The test substance was formulated in polyethylene glycol prior to dosing. Individual animal body weights were recorded prior to test substance administration and then on days 8 and 15. The animals were observed for mortality and clinical signs of toxicity four times on the initial study day and once daily, thereafter, for 15 days. All animals were necropsied at study termination.

All animals dosed at 100 mg/kg survived and gained weight during the study. Signs of toxicity noted during the study included sedation and ruffled fur. No gross internal findings were observed at necropsy.

All animals dosed at 400 mg/kg survived the study. One female lost body weight during the first week of observation, but all animals exceeded their initial body weights by the end of the study. Signs of toxicity noted during the study included sedation, dyspnea, curved body position, emaciation, diarrhea, and ruffled fur. No gross internal findings were observed at necropsy.

At the 600 mg/kg dose level, 1/5 females and 0/5 males died. Decreased body weights were noted in two females and two males during the first week of observation, but all surviving animals exceeded their initial body weights by the end of the study. Signs of toxicity noted in these animals during the study included sedation, dyspnea, curved body position, emaciation, diarrhea, and ruffled fur. At necropsy, the animal that was found dead during the study was noted as having a reddish, discolored liver and filled intestines. No gross internal findings were observed at necropsy for those animals that survived the study.

At the 1000 mg/kg dose level, all animals died by study day 10. Signs of toxicity noted in these animals during the study included sedation, ataxia, dyspnea, curved body position, tremor, emaciation, diarrhea, colorless discharge, and ruffled fur. At necropsy, animals were noted as having discolored lungs and thymus; fluid-filled and yellowish stomachs, cecum, and intestines; and no or severely reduced adipose tissue. Additionally, one animal was cannibalized.

At the 5000 mg/kg dose level, all animals died by study day 4. Signs of toxicity noted in these animals during the study included sedation, dyspnea, latero-abdominal position, curved body position, diarrhea, and ruffled fur. At necropsy, animals were noted as having discolored lungs, as well as fluid-filled stomachs and intestines.

Oral $LD_{50}$ Males = 720 mg/kg (95% C.I. = 557-1171 mg/kg)
Oral $LD_{50}$ Females = 678 mg/kg (95% C.I. = 528-1015 mg/kg)
Oral $LD_{50}$ Combined = 702 mg/kg (95% C.I. = 597-893 mg/kg)

Based on the $LD_{50}$ in rats, Dithianon is classified as EPA Toxicity Category III.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.1100; OECD 401) for an acute oral toxicity study in the rat.

### A-2.2 Subchronic Studies

**<u>90-Day Oral Toxicity - Rat (870.3100)</u>**

In a subchronic oral toxicity study (MRID 44092606), Dithianon (92 ± 0.6% a.i.; Batch #: 15C/86) was administered to 10 Sprague-Dawley rats/sex/group in the diet at dose levels of 0, 30, 180, or 1080 ppm (equivalent to 0/0, 2.53/2.97, 14.64/16.32, and 86.66/99.53 mg/kg/day [M/F]) for 90 days. Additionally, 10 rats/sex received the test material in the diet at 1080 ppm for 90 days and then were allowed a recovery period of 4 weeks.

No adverse treatment-related effects on mortality, clinical signs, food consumption, ophthalmoscopy, auditory acuity, dentition, urinalysis, organ weights, gross pathology, or histopathology were observed.

At 1080 ppm, body weights were decreased ($p<=0.01$) by 10-17% in both sexes during the treatment period, and overall (Weeks 0-13) body weight gains (calculated by reviewers) were decreased by 19-23%. Additionally, terminal body weights were decreased ($p<=0.01$) by 12-18% in both sexes. Slight decreases (8-11%; $p<=0.01$) in hemoglobin, hematocrit, and erythrocytes, as well as increased reticulocytes were noted at Week 13.

In the 1080 ppm recovery group, increases in mean weekly body weight gains (calculated by reviewers) were noted in both sexes during Weeks 14-17, compared to Weeks 10-13, and the Week 17 values for the previously decreased hematology parameters returned to values similar to those seen in the Week 13 controls.

**The LOAEL is 1080 ppm (equivalent to 86.66/99.53 mg/kg/day [M/F]), based on decreased body weight and overall body weight gain in both sexes. The NOAEL is 180 ppm (equivalent to 14.64/16.32 mg/kg/day [M/F]).**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

**<u>90-Day Oral Toxicity - Dog (870.3150)</u>**

In a subchronic oral study (MRID 44092607), Dithianon (92 ± 0.6% a.i., Batch #: 15C/86) was administered to 4 beagle dogs/sex/dose in the diet at nominal doses of 0, 40, 200, or 1000 ppm (equivalent to 0/0, 0.63/0.66, 2.95/3.00, and 12.58/12.61 mg/kg/day [M/F]) for 13 weeks. There were no treatment-related effects on survival, clinical signs, hematology, auditory acuity, ophthalmoscopy, dentition, organ weights, and gross or histopathology.

At 1000 ppm, slight decreases (not statistically significant, NS) in body weights were noted in the females (decr 6-15%) throughout the study, and decreased overall (Weeks 0-13) body weight gains (calculated by reviewers) were observed in both sexes (decr 91-133%). Weekly food consumption was decreased throughout the study in the females (decr 23-49%), attaining statistical significance ($p<=0.01$) at Weeks 1, 4, and 5. Sporadic decreases (not statistically significant, NS) in food consumption were also noted in the 1000 ppm males (decr 5-25%) throughout the study. Mean overall (Weeks 1-13) weekly food consumption (calculated by reviewers) was decreased in the males (decr 7%) and females (decr 31%). Treatment-related clinical chemistry findings were limited to increases ($p<=0.01$) in alkaline phosphatase activity in the males (inc 389-533%) and females (incr 346-477%) at Weeks 6 and 13.

No adverse effects were noted in any parameter at 40 or 200 ppm in either sex.

**The LOAEL for this study is 1000 ppm (equivalent to 12.58/12.61 mg/kg/day [M/F]), based on decreased body weight in females, decreased overall body weight gains and food consumption in both sexes, and increased alkaline phosphatase activity in both sexes. The NOAEL is 200 ppm (equivalent to 2.95/3.00 mg/kg/day [M/F]).**

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3150; OECD 409) for a 90-day oral toxicity study in the dog.

### A-2.3 Developmental Studies

Prenatal Developmental Toxicity Study - Rat (870.3700a)

In a developmental toxicity study (MRID 44092611), Dithianon technical (91.6% a.i.; Batch #: 15C/86) was administered daily via oral gavage in 1% aqueous carboxymethyl-cellulose to 25 or 32 presumed pregnant Sprague-Dawley rats/dose at dose levels of 0 (32 rats), 20 (25 rats), 70 (32 rats), or 100 (25 rats) mg/kg/day in a volume of 10 mL/kg from gestation day (GD) 6 through 15. Approximately 2 months later, an additional 25 dams/dose were treated similarly at dose levels of 0 or 50 mg/kg/day. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

At $\geq$ 50 mg/kg/day, maternal body weights were decreased by 6-18% during GDs 9-20; decreased maternal body weight gains ($p \leq 0.05$) were observed throughout the treatment period (↓43-94% on GDs 6-16) and during the entire gestation period (↓15-26% on GDs 0-20). Decreased food consumption ($p \leq 0.01$) was also noted in these groups throughout the treatment period (↓27-48% on GDs 6-16) and during the entire gestation period (↓13-29% on GDs 0-20). The most common clinical observations were a thin appearance and low food consumption noted in dams at $\geq$ 70 mg/kg/day and red/black vaginal discharge and “few or no feces” observed in dams at 100 mg/kg/day.

In the 100 mg/kg/day group, 5/25 dams died between GD 13 and 17. Four of these 100 mg/kg/day dams that died were thin for two or more days, with low food consumption, pale appearance, and/or red/black vaginal discharge on one or more days. The remaining 100 mg/kg/day dam that died was thin for 8 days, was sluggish the day before death, and had diarrhea on two days prior to death. In the 70 mg/kg/day group, 1/32 dams was killed moribund on GD 14; this dam was thin, with a crusted nose and red/black anal discharge on 1-2 days.

Grossly, an increased incidence of a greyish layer in the gastric wall near the cardia was observed at 70 and 100 mg/kg/day. Additionally, at 100 mg/kg/day, gross pathology findings included increased incidences of mucous layer in the gastric wall.

**The maternal LOAEL is 50 mg/kg/day, based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 20 mg/kg/day.**

Increased incidences of complete litter resorptions (total litter loss) and mean early resorptions per litter were observed in dose groups ≥50 mg/kg/day, compared to concurrent controls. At ≥50 mg/kg/day, the incidence of complete litter resorptions was 20-42%, compared to 0% in controls. This increase in resorptions resulted in a significant increase in post-implantation loss at ≥50 mg/kg/day when all dams surviving until scheduled sacrifice (including dams with whole litter losses) were considered. A significant increase in post-implantation loss was also noted at 100 mg/kg/day when dams with whole litter losses were not considered. Fetal weights, from those dams that survived to scheduled termination with live fetuses, were decreased at 100 mg/kg/day. No apparent dose-response was seen regarding the incidences of variations or malformations observed in this study; however, historical data were not provided for a more comprehensive assessment. The inclusion of data from a second control group and an additional dose group (50 mg/kg/day), which were not run concurrently with the main study, complicated the dose-response assessment of study findings. The registrant should provide dated historical control data, including all anomalies and cesarean data for a period ± two years from the date of the submitted study.

**The developmental LOAEL is 50 mg/kg/day, based on increased incidences of total litter loss and post-implantation loss due to early resorptions. The developmental NOAEL is 20 mg/kg/day.**

This study is classified **acceptable/guideline** and satisfies the guideline requirement **(OPPTS 870.3700a)** for a developmental study in the rat.

## Prenatal Developmental Toxicity Study - Rabbit (870.3700b)

In a developmental toxicity study (MRID 44092613), Dithianon technical (91.6% a.i., Batch #: 15C/86) was administered daily via oral gavage in 1% aqueous carboxymethyl cellulose to 20 presumed pregnant New Zealand White rabbits/dose at dose levels of 0, 10, 25, or 40 mg/kg/day in a volume of 10 mL/kg from gestation day (GD) 6 through 18. Dose levels were based on those used in a range-finding study (MRID 44092614). All surviving does were killed on GD 28; their fetuses were removed by cesarean section and were subjected to external, soft tissue, and skeletal examinations.

**The assessment of maternal toxicity was confounded by incorrect study methodology**.

There were no apparent treatment-related maternal mortalities. The testing facility stated that 5 mortalities (1 at 10 mg/kg/day, 2 at 25 mg/kg/day, and 2 at 40 mg/kg/day) were due to improper gavage technique**,** as evidenced by respiratory tract inflammation/lung findings. There were 6 additional deaths (3 at 10 mg/kg/day, 1 at 25 mg/kg/day, and 2 at 40 mg/kg/day) that were considered to be unrelated to dose, and neither clinical observations nor necropsy revealed treatment-related findings. Since the authors did not note any treatment-related findings at necropsy, since no disease was identified in these animals, and since gavage error is sometimes difficult to document via limited tabulated necropsy data, it is possible that these six deaths were also associated with dosing error or related trauma.

One control doe aborted and was killed on GD 25, with pathological findings in the trachea and abdominal organs at necropsy that indicate that this abortion might also be associated with dosing error. One 10 mg/kg/day doe aborted and was killed on GD 21, and a total of three 40 mg/kg/day does aborted and were killed on GD 18, 19, or 22. No relevant treatment-related pathological findings were observed in these does at necropsy. In the absence of such findings, the assessment of abortion in this study (as a manifestation of maternal toxicity caused by exposure to dithianon) may have been confounded by dosing errors/excessive dosing trauma. This is further supported by the finding of gavage error/trauma associated with abortion and maternal deaths in the range-finding study (MRID 44092614).

At 40 mg/kg/day, maternal body weights were reduced (NS) on GDs 9, 12, and 15 by approximately 5, 6, and 8%, respectively. On GD 28, body weights were decreased by 4-6% in all treated groups, compared to controls, but these differences are neither dose-related nor biologically significant in rabbits. No statistically significant changes in body weights were noted at any time. During the treatment period, a minimal decrease in body weight gain was observed at 10 mg/kg/day (↓4%); however, greater decreases were observed in the 25 (↓48%) and 40 (↓58%) mg/kg/day groups. Overall body weight gain was decreased by 30% in both the 25 and 40 mg/kg/day groups. These apparently large differences in body weight gain result from very small differences in maternal body weight (*i.e.*, 200-300 grams) in 4 to 4.5 kg rabbits, such that the decreases do not reflect toxicologically significant adverse effects.

In this study, the range of food consumption values varies by less than 100 grams. Typically, rabbit food consumption is quite variable and much higher than the ranges reported here. Ranges within 100 g are extremely unlikely for 4.5 kg rabbits. Hence, the apparently large decreases in food consumption, as calculated by reviewers, are neither statistically nor biologically relevant and do not reflect maternal toxicity. In addition, the frequency of these food consumption measurements does not coincide with the study protocol, and there are no protocol amendments presented. (See discussion under the sections on “Compliance” and “Maternal observations and evaluations”).

The maternal LOAEL and NOAEL could not be determined in this study due to methodology problems/issues apparent in the submitted study report that confound interpretation of maternal toxicity.

Pre-implantation loss was 50-94.7% in 28 of the 80 animals placed on the study (including both control and dosed groups); of these, 11 died, likely due to dosing error. Therefore, in actuality, 28 of the 69 control and treated animals on study (41%) had severe levels of pre-implantation loss well outside of any historical control data range. Mean pre-implantation losses per litter were 43.7, 37.9, 32.1, and 57.6 % in the 0, 10, 25, and 40 mg/kg/day dose groups, respectively, which is very excessive. Implantation should be completed prior to the beginning of the dosing period. Instead, it appears that the animals were intubated before the implantation process was finished. Dosing prior to the completion of this process interferes with implantation itself, confounding the interpretation of developmental toxicity. Although a slight increase in pre-implantation loss was apparent at the highest dose tested, this finding is not associated with toxicity of the test material since pre-implantation loss was higher than expected in the control group. The excessive pre-implantation loss was likely the result of methodology error.

At 40 mg/kg/day, when all does that survived to scheduled sacrifice were included, abortions were increased (3 treated vs 1 control; not statistically significant [NS]), and the total number of live fetuses/doe was decreased (3.1 treated vs 6.4 controls; $p \leq 0.05$). Increased early resorptions/doe (2.6 treated vs 0.5 controls) and increased total resorptions/doe (3.0 treated vs 0.7 controls; $p \leq 0.05$) were also noted at this dose. Additionally, increases in complete litter resorptions (2 treated vs 1 controls) and post-implantation losses (51.2% treated vs 13.9% controls; $p \leq 0.05$) were observed.

At 40 mg/kg/day, when does with whole litter losses were excluded, a decreased total number of live fetuses/doe (3.7 treated vs 6.9 controls), increased early resorptions/doe (2.2 treated vs 0.5 controls), and increased total resorptions/doe (2.7 treated vs 0.7 controls; $p \leq 0.05$) were still observed. Given these effects, an increase in post-implantation loss was still noted (41.5% treated vs 7.7% controls), even when whole litter losses were excluded from the calculations. However, it cannot be determined whether the increase in post-implantation loss at the highest dose tested is associated with dosing error/trauma or with test material toxicity, based on the previously identified methodology problems.

There was an apparently dose-dependent effect on the sex ratio, with an increase of 65.9% males at 40 mg/kg/day. An increase was also apparent in the range finding study, which supports this finding. Although the impact of the noted methodology errors on this effect can not be determined, a treatment-related change in sex ratio would be very rare.

There were no effects of treatment on the number of corpora lutea/doe or late resorptions/doe. No effect on mean fetal weights was apparent in any dose level, but since there was an increase in pre-implantation loss, compared to controls and the other treatment groups, the live litter size at the high dose level was also reduced. Because it is known that fetal weights are affected by changes in litter size, assessment of fetal weight data in this study is compromised.

An increased incidence of a number of variations on a litter basis were noted in this study. However, due to the extensive number of dosing errors/trauma and other methodology/GLP issues reported in this study, it is the conclusion of this reviewer that interpretation of potential fetal/developmental toxicity has been confounded. It is unclear whether the observed effects are associated with stress caused by methodology error or if they are due to the toxicity of the test compound.

**A developmental LOAEL and NOAEL could not be determined in this study. Excessive pre-implantation loss in all control and treated groups indicates that animals in this study were likely dosed prior to the completion of the implantation process. High levels of pre-implantation loss reduce live litter size and alter fetal body weights and numerous other potential endpoints of developmental toxicity. As a result, interpretation of fetal wastage and other developmental toxicity data associated with exposure to the test material becomes confounded. In addition, extensive gavage errors caused excessive**

**trauma/stress to the does, further compromising the assessment of fetal/developmental toxicity in this study. Finally, as a result of excessive maternal mortality, too few litters were available to meet guideline requirements, and the power of the statistical assessment to detect potential developmental toxicity effects was reduced.**

This study is classified as **unacceptable/guideline** and does **not** satisfy the guideline requirements **(OPPTS 870.3700b)** for a developmental study in the rabbit.

This study cannot be upgraded. Serious GLP/methodology issues were apparent in the study report, which confounded interpretation of the study data.

### A-2.4 Reproductive Toxicity

**Reproduction and Fertility Effects - Rat (870.3800)**

In a two-generation reproduction toxicity study (MRID 44092615), Dithianon technical (91.6% a.i; Batch #: 15C/86) was administered continuously in the diet to Crl:CD (SD) BR rats (28 rats/sex/dose) at dose levels of 0, 35, 200, or 600 ppm (equivalent to 0/0, 2.2/2.5, 12.6/14.5, and 37.8/42.7 mg/kg bw/day [M/F]). The P and $F_1$ parents were dosed for 100 days before they were mated to produce the $F_1$ and $F_2$ litters, respectively. The $F_1$ pups were weaned on postnatal day (PND) 21, and 25 pups/sex/group were randomly selected as parents of the $F_2$ generation.

There were no treatment-related effects on survival, clinical signs, gross pathology, or histopathology.

During the pre-mating and mating periods, body weights were decreased by 5-6% ($p<=0.05$) compared to controls at 600 ppm in the P generation males, beginning on Day 43 and continuing throughout the remainder of this interval. Additionally in this dose group, body weight gains were decreased by 6-25% ($p<=0.05$) on Days 1-8, 15-22, and 36-43; body weight gains for the overall (Days 1-99) pre-mating period were decreased by 8% ($p<=0.01$), compared to controls. Food consumption was decreased by 6-19% ($p<=0.05$) in these animals beginning on Day 8 and continuing throughout the remainder of pre-mating, with the exception of Days 69-71 and 76-78. In the P females and $F_1$ males, there were no adverse effects of treatment on body weights, body weight gains, or food consumption during the pre-mating and mating periods. In the $F_1$ females, food consumption was frequently decreased by 9-18% ($p<=0.05$) at 600 ppm during pre-mating. However, body weights were only minimally decreased (decr. 6-7%; $p<=0.05$) in this group on pre-mating days 15, 22, and 29. Body weight gain was decreased by 10% ($p<=0.01$) in these animals for Week 1 (Days 1-8), but overall body weight gain for pre-mating was comparable to controls.

During the gestation and lactation periods, there were no effects of treatment on body weights, body weight gains, or food consumption in the parental females of either generation.

**The LOAEL for parental toxicity is 600 ppm (equivalent to 37.8/42.7 mg/kg/day [M/F]), based on decreased body weights, body weight gains, and food consumption during pre-mating. The NOAEL is 200 ppm (equivalent to 12.6/14.5 mg/kg/day [M/F]).**

There were no effects of treatment on gestation duration, the number of implantations, sex ratio, or on live birth, weaning, viability, or lactation indices. Litter weights and pup body weights of the treated groups were comparable to controls throughout the post-natal period in both generations. No clinical signs were reported.

**The LOAEL for offspring toxicity was not observed. The NOAEL is 600 ppm (equivalent to 37.8/42.7 mg/kg/day [M/F]).**

There were no treatment-related effects on any reproductive parameter in either generation. Pre-coital interval and insemination, fecundity, fertility, and gestation indices of the treated groups were comparable to controls. Weights of the testes and epididymides in the treated males were comparable to controls.

**The LOAEL for reproductive toxicity was not observed. The NOAEL is 600 ppm (equivalent to 37.8/42.7 mg/kg/day [M/F]).**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

### A-2.5 Chronic/Carcinogenicity Studies

<u>**Chronic Toxicity - Dog (870.4100b)**</u>

In a chronic oral toxicity study (MRID 44092608), 4 beagle dogs/sex/dose were given Dithianon (92% a.i., Batch #: 15C/86) in the diet at nominal doses of 0, 40, 200, or 1000 ppm (equivalent to 0/0, 1.5/1.6, 6.7/7.6, and 37.1/35.0 mg/kg/day [M/F]) for up to 52 weeks.

No adverse treatment-related effects were observed in mortality, clinical signs, body weight, body weight gain, food consumption, ophthalmoscopy, urinalysis, or gross pathology.

In the liver, absolute and relative organ weights were increased at 1000 ppm in both sexes, and minimal to moderate hypertrophy was observed in all 1000 ppm males and all >=200 ppm females. Additionally, minimal to slight histiocyte pigment (which tested positive for iron) was observed at 1000 ppm in 3 animals/sex. In the 1000 ppm females, decreases ($p<0.05$) in hemoglobin, erythrocytes, and hematocrit were observed throughout the study. Additionally at 1000 ppm, alkaline phosphatase was increased ($p<0.05$) in both sexes at Weeks 26 and 52, and blood urea nitrogen was decreased ($p<0.05$) in the males at Week 26 and in the females throughout the study.

In the kidney, absolute and relative organ weights were increased at 1000 ppm in both sexes; minimal to slight pigmentation was observed in the >=200 ppm females (3-4 treated vs 1 control); minimal pigment was observed in the 40 and 200 ppm males (3-4 treated vs 4 controls); and minimal (3/4 treated) to slight (1/4 treated) pigment was observed in the 1000 ppm males. At 1000 ppm, this finding was increased in incidence and in severity in the females, while it was only increased in severity in the males.

Additional findings in the 1000 ppm males were limited to increased trends ($p<0.05$) in total leukocytes at Weeks 26 and 52 and in neutrophils and lymphocytes at Week 52.

The only finding at 200 ppm was an increased incidence of renal pigmentation in the females, but this was not corroborated with increased kidney weight except at 1000 ppm.

**The LOAEL is 1000 ppm (equivalent to 37.1/35.0 mg/kg/day [M/F]), based on increased absolute and relative liver and kidney weights, increased alkaline phosphatase, decreased blood urea nitrogen, hepatocellular hypertrophy, histiocyte pigmentation, and renal pigmentation in both sexes, as well as decreased hematology parameters (hemoglobin, erythrocytes, and hematocrit) in the females. The NOAEL is 200 ppm (equivalent to 6.7/7.6 mg/kg/day [M/F]).**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

__Carcinogenicity Study - Mouse (870.4200b)__

In a carcinogenicity study (MRID 44092609), Dithianon (92% a.i.; Batch #: 15C/86) was administered in the diet to Crl:CD-1(ICR)BR mice (51/sex/dose) at doses of 0, 20, 100, or 500 ppm for up to 18 months. These doses were approximately equivalent to 0, 2.9, 14.3, and 71.4 mg/kg/day (estimated by the reviewers).

No treatment-related effects were observed on body weights, body weight gains, food or water consumption, differential leukocyte counts, or gross pathology.

Decreased survival was observed in the 500 ppm males. At Week 65, survival was 78.4% at 500 ppm vs 90.2% in each other group. At Week 80, survival in the dose groups was as follows: 74.5% (20 ppm), 70.6% (100 ppm), and 62.7% (500 ppm) vs 72.5% in controls. There was an increasing trend ($p<=0.05$) in mortality with dose, but the treated groups did not differ ($p>0.05$) from the controls as determined by pairwise statistical testing.

Nephrotoxicity was evident at 500 ppm. Increased ($p<=0.05$) kidney weights were observed in both sexes (relative to body: incr 40-54%; absolute: incr 35-42%). Increased incidences of the following lesions (# affected/51) were observed in the kidney: (i) chronic nephropathy, minimal to moderately severe in 45 males vs minimal in 31 controls; minimal to moderately severe in 37 females vs minimal to moderate in 20 controls; (ii) cortical cyst(s), minimal to severe in 12 males vs minimal to moderately severe in 5 controls; minimal to severe in 10 females vs slight to moderate in 3 controls; (iii) tubular dilatation containing flocculent material, minimal to severe in 42 males vs 0 controls; minimal to severe in 40 females vs minimal to slight in 3 controls; and (iv) infarct(s), minimal or moderately severe in 7 males vs moderate in 1 control; minimal to moderately severe in 7 females vs slight or moderately severe in 2 controls. Additionally, it was stated that the incidence of fur staining was increased from about Week 9 in both sexes.

**The LOAEL is 500 ppm (approximately equivalent to 75 mg/kg/day, estimated by reviewers), based on increased mortality in males, increased kidney weights and increased incidences and severity of kidney lesions (chronic nephropathy, cortical cysts, tubular dilatation, and infarct) in both sexes. The NOAEL is 180 ppm (approximately equivalent to 15 mg/kg/day).**

No treatment-related increase in neoplasia was observed. The carcinogenic potential is considered negative in this study.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

**<u>Combined Chronic Toxicity/Carcinogenicity - Rat (870.4300)</u>**

In this combined chronic toxicity/carcinogenicity study (MRIDs 44092616, 44092617, 44092618), Dithianon (92% a.i., Lot/Batch # 1-15C/86 and 2-15C/86) was administered to 70 Crl:CD (SD)BR rats/sex/group in the diet at dose levels of 0, 20, 120, or 600 ppm (approximately equivalent to 0, 1, 6, and 30 mg/kg/day in males and females [estimated by reviewers]) for up to 104 weeks.

No adverse treatment-related effects were noted on mortality, water consumption, ophthalmoscopy, or hematology.

At 600 ppm, the incidence of fur staining and rough haircoat was generally greater in females than in controls, beginning at Week 13. At this dose, body weights and overall (Weeks 0-104) body weight gains were decreased in both sexes. Weekly food consumption was slightly decreased in males in the first and last few weeks of the study, and in females throughout the study.

At 600 ppm, relative-to-body kidney weights were increased ($p<=0.001$) in both sexes. In the kidney, the incidences of grossly observed irregular surfaces, pale color, cysts, and enlarged size in males, as well as masses in females, were increased. Increased incidences of minimal to moderately severe tubular nephrosis were observed in both sexes. In females, increased incidences of minimal to moderately severe proliferative tubules and minimal to moderately severe glomerulonephropathy were observed. In males, increased incidences of renal cysts, end-stage kidney lesions (characterized by extensive cortical destruction, cysts, and fibrosis), and increased urine volume ($p<=0.001$) were observed. Although no treatment-related effects on the overall mortality rates were observed, the incidence of morbidity and mortality specifically due to renal and urogenital tract lesions was higher in the 600 ppm males. Additionally, increased incidences of aortic mineralization, parathyroid hyperplasia, and arteritis of the pancreas and testes observed in males were considered by the Sponsor to be secondary responses to treatment-related chronic renal damage.

The results from a concurrently submitted mechanistic study (MRID 44092617) demonstrate that dithianon induced hydropic degeneration with islands of basophilic tubules and increased renal cell proliferation. These effects are indicative of active regeneration of damaged tissue and suggest a potential correlation between renal damage, repair, and tumor formation. Additionally, the results from an additional concurrently submitted investigational study (MRID 44092618) suggest that mitochondrial damage may be a contributing factor in dithianon-induced kidney damage.

At the lower doses in females, there was a dose-dependent increase in the incidence and severity of minimal to moderately severe glomerulonephropathy. Given the incidence in control animals and the dose-related increase, this effect likely reflected a treatment-related exacerbation of an age-related phenomenon.

**The LOAEL is 600 ppm (approximately equivalent to 30 mg/kg/day) based on decreased body weight gain and increased relative-to-body kidney weights in both sexes, grossly observed kidney lesions in males (irregular surfaces, pale kidneys, cysts, and enlarged kidneys) and females (masses), and non-neoplastic lesions of the kidney in males (tubular nephrosis, renal cysts, and end-stage kidney lesions) and females (tubular nephrosis, proliferative tubules, and glomerulonephropathy). The NOAEL is 120 ppm (approximately equivalent to 6 mg/kg/day).**

At the doses tested, there was a treatment-related increase in benign neoplasms of the kidney in females compared to controls. At Week 104, there was an increase in renal adenomas at 600 ppm in females (14% treated vs 0% controls). One animal had both an adenoma and a carcinoma, and a single animal had a renal carcinoma only. No statistical analysis or historical control data were reported by the Sponsor. Dosing was considered adequate based on decreased body weight gain, increased kidney weight, and gross and microscopic lesions of the kidney.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

### A-2.6 Mutagenicity Studies

<u>**Bacterial Gene Mutation (870.5100)**</u>

***STUDY 1***

In independently performed reverse gene mutation assays in bacteria (MRID 44092619, Study 1; MRID 44280401, Draft QA/QC Study Report), strains TA1535, TA1537, TA1538, TA98, and TA100 of *S. typhimurium* were exposed to Dithianon (90% a.i.; Batch No. 15C/86), in dimethyl sulfoxide, DMSO, at concentrations ranging from 0.1 to 333.3 µg/plate in the absence of mammalian metabolic activation and 10 to 3333.3 µg/plate in the presence of mammalian metabolic activation. The S9 cofactor mix was derived from the livers of male Wistar rats induced with Aroclor 1254. Concentrations used in these assays were based on the findings from Study LMP 200A (listed as Study No.2 in this DER). From the finding of a preliminary cytotoxicity in Study No. 2, compound precipitation and cytotoxicity were seen at 5000 µg/plate.

Cytotoxicity was only noted at the highest dose tested (HDT) in strain TA1537 (66.6 µg/plate -S9). The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of a concentration-related positive response of induced mutant colonies over background.**

When used in conjunction with the data from Study No.2 , this study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

***STUDY 2***

In independently performed reverse gene mutation assays in bacteria (MRID 44092619, Study 2; MRID 44280402, Draft QA/QC Study Report), strains TA1535, TA1537, TA1538, TA98, and TA100 of *S. typhimurium* were exposed to Dithianon (90% a.i.; Batch No. 15 C/86), in dimethyl sulfoxide, DMSO, at concentrations ranging from 1 to 333.3 µg/plate in the absence of mammalian metabolic activation and 33.3 to 3333.3 µg/plate in the presence of mammalian metabolic activation. The S9 cofactor mix was derived from the livers of male Wistar rats induced with Aroclor 1254. Based on the findings of the preliminary cytotoxicity assay, compound precipitation was seen at 5000 µg/plate.

Cytotoxicity was noted at the highest dose tested (HDT) in all strains (5000.0 µg/plate +/-S9). The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of a concentration-related positive response of induced mutant colonies over background.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

**<u>Mammalian Gene Mutation (870.5300)</u>**

***STUDY 3***

In independently performed mammalian cell gene mutation assays (MRID44092619, Study 3; MRID 44280403, QA/QC Draft Study Report), Chinese hamster lung V79 cells cultured *in vitro* were exposed to Dithianon (Unknown purity; Batch No. 30/84), in dimethyl sulfoxide (DMSO), at concentrations of 0, 20.0, 50.0, 100.0, or 200.0 µg/mL in the absence and 60.0, 150.0, 300.0, or 600.0 µg/mL in the presence of mammalian metabolic activation in both trials for 4 hours. The S9 cofactor mix was derived from the livers of male Wistar rats induced with Aroclor 1254.

Dithianon was not tested to adequate cytotoxic concentrations; relative plating efficiencies at 200 µg/mL-S9 were >30 or >18% in trials 1 or 2, respectively, and >50% at 600 µg/mL +S9 in both trials. The positive controls induced the appropriate response. **There was no evidence of a concentration-related positive response of induced mutant colonies over background.**

This study is classified as **unacceptable/guideline** and does not satisfy the guideline requirements (OPPTS 870.5300; OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data because of the lack of adequate cytotoxicity at the HDT. Additionally, no purity information or other characteristics that define the test substance were provided.

***STUDY 4***

In independently performed mammalian cell gene mutation assays (MRID 44092619, Study 4; MRID 44280404, QA/QC Draft Study Report) Chinese hamster lung V79 cells cultured *in vitro* were exposed to dithianon (90%, Batch No. 15 C/86) in DMSO, at concentrations of 0, 0.03, 0.56, 1.00 or 1.33 µg/mL in the absence and 0, 0.33, 0.56, 1.00, or 1.33 µg/mL in the presence of mammalian metabolic activation (Trial 1); 0, 0.33, 0.56 or 1.00 µg/mL -S9 or 0, 0.33, 1.00 or 1.33 µg/mL +S9 (Trial 2); 0, 0.10, 0.33, 0.56, 1.00 or 1.33 µg/mL +S9 (Trial 3); 0, 0.03, 0.10, 0.33 0.56 or 1.00 µg/mL -S9 or 0.10, 0.33, 0.56, 1.00 or 1.33 µg/mL +S9 (Trial 4) for 2 hours. The S9 cofactor mix was derived from the livers of male Wistar or Sprague-Dawley rats induced with Aroclor 1254.

In the preliminary cytotoxicity assessment, no cells survived treatment with Dithianon concentrations 3.3 µg/mL -/+S9; compound precipitations was also noted at 33 µg/mL +/-S9. At 1.0 µg/mL, the cloning efficiency was 3% -S9 and 15% +S9. In the mutation assays, there were no reproducible or dose-related increases in the mutation frequencies (MFs) induced by the nonactivated test material. However, S9-activated dithianon induced a reproducible, concentration-related and >3-fold increases in the MF in Trials 3 and 4. In Trial 4, fold increases were 3.7-, 5.7- and 10.5 over control at 0.56, 1.00 and 1.33 µg/mL, respectively, MFs at these levels were 2.2, 3.4 and 6.3 x $10^5$ vs. 0.6 x $10^5$ for the solvent control and CEs were 137, 55 and 12%, respectively. The positive controls induced the appropriate response. Although there was evidence of a concentration related increase in the MF, the relevance of these findings is questionable. Considering the wide range of spontaneous MF for V79 cells (0-100 x $10^{-6}$ clonable cells), the mammalian cell gene mutation assays working group (Aaron *et al.*, 1994) developed a consensus cutoff MF (>20 x 10-6) that must be met in order to classify a positive response in the V79 mammalian cell forward gene mutation assay. This response must be accompanied by an absolute increase in mutant colonies and a dose response. Using these most recent consensus criteria, the V79 cell assay results with dithianon with spontaneous MFs ranging from 0.05 to 0.20 x$10^{-6}$ while acceptable are nevertheless very low. Similarly, MFs for the treatment groups range from a low of 0.04 to a high of 1.36 x$10^{-6}$ (-S9) or from a low of 0.04 to a high of 0.87 x$10^{-6}$ (-S9). Although increases were seen, none of these treatment values matches or exceeds the cutoff MF required to call the test material positive. **It was, therefore, concluded that there is insufficient evidence that dithianon is mutagenic in this test system.**

This study is classified as acceptable (guideline) and satisfies the guideline requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

**<u>*In Vitro* Mammalian Chromosome Aberration Test (870.5375)</u>**

In independently performed mammalian cell cytogenetic assays (chromosome aberration) (MRID 44092620, Study 1; MRID 44280405, Draft QA/QC Study Report), Chinese hamster lung fibroblasts (V79) cells were exposed to Dithianon technical (91.6% a.i.; Batch No. 15 C/86), prepared in dimethyl sulfoxide (DMSO) in the absence and the presence of S9 activation (Aroclor 1254-induced male Wistar rat livers) at concentrations and fixation times of:

| **Concentrations** | | **Fixation Times** |
|---|---|---|
| 0 or 600 ng/mL-S9 | 0, 5000 ng/mL+S9 | (7 hours) |
| 0, 25, 50, 600 ng/mL-S9 | 0, 500, 1000, 5000 ng/mL+S9 | (18 hours) |
| 0 or 300 ng/mL-S9 | 0, 3500 ng/mL+S9 | (28 hours) |

The S9 liver fraction was derived from the livers of Wistar rats induced with Aroclor 1254.

Dithianon technical was cytotoxic at the highest nonactivated concentration (600 ng/mL -S9), reducing the plating efficiency (PE) to 13.3% of control, and also at the highest S9-activated concentration (5000 ng/mL +S9), reducing the PE to 40.5% of control. Neither concentration had an appreciable cytotoxic effect on the mitotic index. At the 7-hour harvest time, increases in the incidence of aberrant cells were seen at 600 ng/mL -S9 (10.0 or 13.5% aberrant cells vs 1.0% in the solvent control cultures of both trials) and at 5000 ng/mL +S9 (8.0 or 10.5% aberrant cells vs 1.0 % in the solvent control cultures of both trials). At the 18-hour harvest, a marked clastogenic effect was seen in the second trial at 600 ng/mL -S9 (16.5% aberrant cells vs 1 or 2% in the negative and solvent control cultures); the response was confined to this level. With S9-activation, reproducible increases were scored only at 5000 ng/mL (26.5 % aberrant cells vs 2.0% in the solvent control cultures–Trial 1 and 16.5% aberrant cells vs 2.5% in the solvent control cultures–Trial 2). Results for lower concentrations were negative. Cells harvested 28 hours after treatment with 300 ng/mL -S9 were negative for structural chromosome aberrations, but there was a marked increase in aberrant cells (15.5% aberrant cells vs 0.5% in solvent control cultures) at 3500ng/mL (Trial 1) and a slight (6.5% aberrant cells vs 0.0 in solvent control cultures) at this concentration in Trial 2. Chromatid-type breaks and/or fragments were the most frequently scored chromosome aberrations at the 7-hour harvest interval. However, at 18 hours, a marked increase in chromosome exchanges was scored in cells treated with 600 ng/mL -S9 in one of the two trials and in both trials for cells exposed to 5000 ng/mL +S9. Marked increases in chromosome exchange figures also were noted in cells treated with 3500 ng/mL +S9 and harvested at 28 hours. The positive controls, evaluated only at the 18-hour harvest interval, induced the expected clastogenic effect.

**There was *evidence* of structural chromosome aberrations induced over background.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5375; OECD 473) for *in vitro* cytogenetic mutagenicity data**.**

<u>**Mammalian Bone Marrow Chromosomal Aberration Test (870.5385)**</u>

In an <u>in vivo</u> bone marrow cytogenetic assay (MRID 44092620, Study 2; MRID 44280406, Draft QA/QC Study Report), groups of six male and six female Wistar rats received single oral gavage administrations of 22.3, 106.0, or 393.5 mg/kg Dithianon technical (91.6% a.i.; Batch No. 15C/86). The test material was delivered to the animals as suspensions prepared in polyethylene glycol (PEG) 400. Animals were sacrificed 6, 24, and 48 hours following compound administration, and bone marrow cells from ten animals per group (5 males and 5 females) were harvested and examined for the incidence of structural chromosome aberrations.

Overt toxicity was manifested as reduced spontaneous activity, eye closure, and apathy in all high-dose animals. There was no evidence of a cytotoxic response in the target organ. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There was, however, no indication that Dithianon technical induced a clastogenic effect at any dose or sacrifice time.**

The study is classified as **acceptable/guideline** and satisfies the requirements for FIFRA Test Guideline 84-2 for <u>in vivo</u> cytogenetic mutagenicity data.

**Mammalian Erythrocyte Micronucleus Test (870.5395)**

In a NMRI mouse bone marrow micronucleus assay (MRID 44092620, Study 3; MRID 44280407, QA/QC Draft Study Report), five mice/sex/dose were treated with a single oral administration of Dithianon technical (unspecified purity; Batch No. 162/83) at doses of 0, 1, 10, or 100 mg/kg bw. Bone marrow cells were harvested at 24, 48, and 72 hours post-treatment. The vehicle was 0.5% carboxymethyl cellulose (CMC).

Reduced activity, dyspnea, ptosis, and diarrhoea were seen at the highest dose tested (100 mg/kg). The positive control induced the appropriate cytogenetic response. **However, there were no significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **unacceptable/guideline** and does not satisfy the guideline requirements (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data because information on the purity of the test material was not provided. The study can, however, be upgraded if the missing information is provided.

**Unscheduled DNA Synthesis in Mammalian Cells in Culture (870.5550)**

In an unscheduled DNA synthesis assay (MRID 44092621), primary rat hepatocyte cultures were exposed to Dithianon Technical (90%, a.i.; Batch No. 15 C/86), in dimethyl sulfoxide (DMSO), at concentrations of 0, 0.1, 1.0, 5.0, 10.0, 15.0, or 20.0 µg/mL for 3 hours. Nuclear DNA was isolated quantitatively, and the incorporation of radiolabeled thymidine ([$^3$H]-TdR) was measured by liquid scintillation counting (LSC).

In the preliminary cytotoxicity test, no cells survived treatment with 25.0 µg/mL; no cytotoxic effects were seen in the main assay up to the highest concentration tested (20 µg/mL). The positive control induced the expected marked increase in UDS. **There was, however, no evidence that Dithianon technical induced a genotoxic response up to the highest subcytotoxic dose assayed.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5550; OECD 482) for other genotoxic mutagenicity data.

**A-2.11 Metabolism - Rat (870.7485)**

**Metabolism and Pharmacokinetics - Rat (870.7485)**

In a series of rat metabolism studies (MRIDs 44092622 and 44092623), [$^{14}$C]-dithianon (Batch # CH. 9166; radiochemical purity >97%) in 1.0% (w/v) carboxymethylcellulose, 4% dextrose (or glucose), 0.18% (w/v) saline solution was administered to Sprague-Dawley rats by gavage. The compound was administered as a single gavage dose at 10 or 50 mg/kg nominal in a mass balance/tissue distribution study (five rats/sex/dose), bile duct cannulation study (three rats/sex/dose), plasma pharmacokinetics study (five rats/sex/dose), tissue distribution study (five/sex treated with 10 mg/kg only), and biliary metabolism study (one/sex treated with 50 mg/kg only). In addition, non-radiolabeled compound was administered to six rats/sex as a single daily gavage dose at 10 mg/kg nominal for 14 consecutive days followed by a radiolabeled dose (also at 10 mg/kg nominal) on Day 15. Bile, urine, feces, liver, and kidneys were analyzed to determine metabolic profiles. Also, [$^{14}$C]-dithianon was administered as a single daily gavage dose for seven days at 10 mg/kg to five males, and an additional male received a single dose and was terminated 24 hours later. Six sagittal sections though the carcass were prepared from each of these animals and analyzed by autoradiography to determine tissue distribution qualitatively.

In both sexes (M&F), doses (10 & 50 mg/kg), and single as well as repeated dose groups (where applicable), overall recovery of the radioactive dose was 95.8-99.3% after 120 h in the mass balance/tissue distribution studies (both single and repeated doses). Absorption was rapid, as radioactivity was detected in plasma within 15 minutes (first time point analyzed), with maximum plasma concentrations being achieved within six hours. At least 31-43% of the administered dose is absorbed, as this amount was isolated in the urine and bile. Absorption was not dose limited at 50 mg/kg, as there was a proportional increase in the AUC values from analyzed plasma from the 10 to 50 mg/kg groups. By 120 h, the administered dose was recovered predominantly in the feces (64-72% dose) and urine (27-31% dose). Cage wash accounted for <0.7% dose and the tissues/carcass accounted for <=0.2% dose. A preliminary study was performed, which indicated that [$^{14}$C] was not exhaled into the air. Concentrations of radioactivity in specific tissues generally decreased by an order of magnitude each 24 hours for the first three days, indicating no bioaccumulation occurred. The terminal half-life of radioactivity in plasma is 46-57 h. The excretion profile was not affected by sex, dose, or the number of doses. The bile cannulation study provided a similar excretion profile.

The concentration of radioactivity was highest in the kidney in both sexes at all time points (2.01-2.73 µg equiv./g at six h post-dose to 0.127-0.149 µg equiv./g at 168 h post-dose). The liver, plasma, and whole blood had similar concentrations throughout the study and at six h were 0.519-0.757 µg equiv./g. The concentrations of radioactivity in other tissues were comparable to or less than the concentrations in plasma, or they were below the limit of detection throughout the study. Radioactivity was not detected in the brain or spinal cord. No sex-based differences were noted in tissue distribution.

Metabolites were primarily isolated by 1D-TLC. Further purification was accomplished by using 2D-TLC, or purification of particular 1D-TLC fractions with HPLC. The metabolic pattern was generally similar between sexes. Unchanged parent was present in the feces in minor amounts (<=0.5% dose). Results indicated that the parent was extensively degraded. In the urine, 15 metabolite fractions were separated, and 7 metabolite fractions were found in the feces. In addition to parent, two compounds were identified through HPLC-MS as 2-amino-1,4-naphthoquinone and 4,9-dioxo-4,9-dihydronaphtho[2,3-b]thiophene-2,3-dicarbonitrile. The Sponsor also stated that the presence of the penta-fluorobenzyl conjugate of 2-hydroxy-3 mercapto-1,4-napthoquinone could be proposed, and an additional three compounds had the mass doublet characteristic of the naphthalene moiety of dithianon. From the data provided, only one fraction had >5% dose. This fraction occurred in the 8-24 hour urine samples of the 50 mg/kg group and was characterized as a glucuronic acid conjugate. Analysis of kidney and liver extracts (42-61% extraction efficiency) revealed many fractions from the kidney sample and no clearly defined peaks from the liver sample. Analysis of the bile indicated a large proportion of the radioactivity was associated with material that remained at or close to the base line of TLC plates (3.6-4.5% of the administered dose). In bile incubated with β-glucuronidase, there was a small decrease in base line radioactivity, with an accompanying increase in radioactive components with a high relative mobility. All fractions contained <5% of the administered dose.

The data indicated that dithianon appeared to be broken down in the gastrointestinal tract, possibly by the resident gut flora, as only very low concentrations of the unaltered parent compound were identified in the feces.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.7485, OPP 85-1) for a Tier 1 metabolism study in rats.

## A-3.0 METABOLISM CONSIDERATIONS

### A-3.1 TEAM PROPOSAL

In the rat, essentially all of the administered dithianon was rapidly degraded, mainly into a large number of polar products, none of which could be considered a main metabolic product. In plants, dithianon is apparently not highly metabolized; when metabolism does occur, it results in a number of very polar, unidentifiable fragments, none of which are significant. The dithianon risk assessment team concludes that the ROC in plant and ruminant commodities is the parent compound only (dithianon *per se).*

## A-3.2 NATURE OF THE RESIDUE IN PLANTS

Executive Summary of Apple Metabolism Study

American Cyanamid Company (now BASF Corporation) has submitted a study investigating the metabolism of [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}C$]-dithianon (specific activity 51.50 to 51.53 µCi/mg) in apple. The radiolabeled test substance was formulated as a soluble concentrate (SC) and applied as four or five surface applications to individual apples. Each treated apple and associated leaves received 100 µL per application of the formulated solution containing 0.10% dithianon. Mature apple fruit, the raw agricultural commodity (RAC), and leaves were collected 21 days after four treatments and 15 days after five treatments. In addition, apples and leaves were collected following the first application to evaluate run-off. The in-life and analytical phases of the study were conducted by Huntingdon Research Centre Limited (Cambridgeshire, England). Total radioactive residues (TRR), determined by summing extractable and non-extractable radioactivity, were 5.4 ppm in/on fruit and 217 ppm in/on leaves treated with four applications. The TRR were 2.6 and 485 ppm, respectively, in/on apple fruit and leaves treated with five applications.

Surface washes with dichloromethane (DCM) removed the majority of the radioactivity (81 to 94% TRR) from apple fruit and leaves, while a subsequent surface washing with acetone removed less than 3% TRR. Extraction of the separated peel and pulp and leaves with acetone released less than 3% TRR from each matrix. All solvents used for surface washing and extraction contained 0.1% acetic acid to prevent degradation of dithianon. Non-extractable residues accounted for 7.3 to 10.7% TRR in fruit (peel and pulp) and 5.1 to 8.1% TRR in leaves following extraction. Because TRR were determined by summing extractable and non-extractable radioactivity, accountabilities were 100%. Residues were identified by TLC with confirmatory analysis by alternate TLC systems. These methods successfully identified the predominant residues in apples. Apple fruit and leaves were extracted on the day of harvest; the maximum potential storage duration based on the study experimental completion date would have been 8 months from harvest/extraction to analysis. Additional information is required concerning the storage conditions and durations of the surface washes and extracts.

Approximately 70 to 86% TRR were identified in apple fruit and leaves. Dithianon was the only residue identified, accounting for 82.0% TRR (4.43 ppm) and 72.7% TRR (1.89 ppm) in the surface washes of fruit treated with four and five applications, respectively. Dithianon accounted for 69.7% TRR (151 ppm) and 85.7% TRR (415 ppm) in the surface washes of leaves treated with four or five applications, respectively. TLC analysis using neutral solvent systems revealed a baseline region in the surface washes of fruit and leaves which represented 6 to 18% TRR. On TLC analysis using an acidic system, the baseline material was reduced, and some of the radioactivity appeared to co-elute with dithianon. Based on these results, the petitioner concluded that the baseline material represented at least two fractions, which were estimated by the differences in the amounts of the baseline radioactivity detected using the two different TLC systems to account for 0.03 to 0.09 and 0.14 to 0.24 ppm in fruit, and 24 to 29 and 4 to 10 ppm in leaves. The baseline material also remained in the baseline region with reverse-phase TLC analysis of the apple surface wash, suggesting that the degradation product may be a polymeric degradation product rather than a polar component. Residues in the extracts (less than 3% TRR) of fruit and leaves were very polar in nature and did not co-elute with any of the reference standards.

Fruit and leaf samples were also collected following the first application to evaluate run-off of the test solution during and immediately after application. Approximately 90 to 91% of the applied radioactivity was recovered in the surface washes of fruit and leaves, with only 0.3% of the applied radioactivity present in the extracts and 1 to 2% remaining as non-extractable residues. Dithianon was identified as the major residue in the surface washes (91 to 93%).

Based on the results of the apple metabolism study, in conjunction with the orange and wheat metabolism studies associated with DP Barcode D312241 (see DERs for MRIDs 44092625 and 44092626), the petitioner concluded that dithianon is not highly metabolized in plants, with a significant amount of the unchanged parent remaining on the plant surface and little to no movement from the application site. When metabolism does occur, it results in a number of very polar, unidentifiable fragments, none of which are significant.

Tabular Summary of Apple Metabolism Study

**APPENDIX TABLE 3.2.1** **Summary of Characterization and Identification of Radioactive Residues in Apple Matrices Following Application of [$^{14}$C]-Dithianon.**

| Compound | 4 Applications | | | | 5 Applications | | | |
|---|---|---|---|---|---|---|---|---|
| | Fruit | | Leaves | | Fruit | | Leaves | |
| | TRR = 5.4 ppm | | TRR = 217 ppm | | TRR = 2.6 ppm | | TRR = 485 ppm | |
| | % TRR | ppm | % TRR | ppm | % TRR | ppm | % TRR | ppm |
| Dithianon | 82 | 4.43 | 69.7 | 151 | 72.7 | 1.89 | 85.7 | 415 |
| Baseline | 6.1 | 0.33 | 18.2 | 39 | 6.7 | 0.17 | 5.7 | 28 |
| Other | 1.6 | 0.09 | 2.4 | 5 | 4.6 | 0.12 | 2.7 | 8 |
| Acetone surface wash | 0.5 | 0.03 | 0.7 | 2 | 2.8 | 0.07 | 0.2 | 1 |
| Peel and Pulp or Leaf Extract | 2.6 | 0.14 | 1 | 2 | 5 | 0.13 | 0.6 | 3 |
| Total identified | 82 | 4.43 | 69.7 | 151 | 72.7 | 1.89 | 85.6 | 415 |
| Total characterized | 10.9 | 0.59 | 22.3 | 48 | 19.1 | 0.49 | 9.2 | 40 |
| Total extractable | 92.8 | 5.01 | 92 | 200 | 89.2 | 2.32 | 94.8 | 460 |

| APPENDIX TABLE 3.2.1 | Summary of Characterization and Identification of Radioactive Residues in Apple Matrices Following Application of [$^{14}$C]-Dithianon. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **4 Applications** | | | | **5 Applications** | | | |
| | **Fruit** | | **Leaves** | | **Fruit** | | **Leaves** | |
| | **TRR = 5.4 ppm** | | **TRR = 217 ppm** | | **TRR = 2.6 ppm** | | **TRR = 485 ppm** | |
| | **% TRR** | **ppm** | **% TRR** | **ppm** | **% TRR** | **ppm** | **% TRR** | **ppm** |
| Unextractable (PES) [1] | 7.3 | 0.39 | 8.1 | 18 | 10.7 | 0.27 | 5.1 | 25 |
| Accountability [2] | 100 | | 100 | | 100 | | 100 | |

1. Residues remaining after exhaustive extractions; for fruit, includes non-extractable residues of peel and pulp.
2. Accountabilities equal 100% because the TRR was calculated as the sum of radioactivity in the surface washes, extracts and non-extractable solids.

Executive Summary of Orange Metabolism Study

American Cyanamid Company (now BASF Corporation) has submitted a study investigating the metabolism of [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}$C]-dithianon (specific activity 29.82 µCi/mg) in orange. The radiolabeled test substance was formulated as a soluble concentrate (SC) and applied as two spray applications to an orange tree at a rate of 0.35 pounds active ingredient per acre (lb ai/A) per application, for a total seasonal rate of 0.70 lb ai/A. Mature orange fruit, the raw agricultural commodity (RAC), were collected 14 and 28 days after the last application. The in-life phase of the study was conducted by Research for Hire (in Porterville, CA), and the analytical phase was conducted by Cyanamid Forschung GmbH (in Schwabenheim, Germany).

Total radioactive residues (TRR), determined by summing extractable and non-extractable radioactivity, were 4.47 and 5.26 ppm in/on whole oranges harvested at the 14- and 28-day pre-harvest intervals (PHIs), respectively.

Surface washes with dichloromethane (DCM) removed the majority of the radioactivity (87 to 94% TRR) from oranges, and a subsequent surface washing with acetone removed less than 3 to 7% TRR. Extraction of the separated peel (with DCM) and pulp (with acetone) released less than 1 to 5% TRR from each matrix. All solvents used for surface washes and extraction contained 0.1% acetic acid to prevent degradation of dithianon. Non-extractable residues accounted for less than 6% TRR (peel and pulp); additional radioactivity (3.2% TRR) was released from the 28-day PHI peel by enzyme hydrolysis with pronase. Because TRR were determined by summing extractable and non-extractable radioactivity, accountabilities were essentially 100%. Residues were identified by TLC with confirmatory HPLC analysis of the parent. These methods successfully identified the predominant residues in oranges. Supporting storage stability data are not required because all samples were stored frozen and analyzed no more than 5 months after harvest.

Only residues in 28-day PHI oranges were further characterized. Approximately 80% TRR were identified in whole oranges. Dithianon was the only residue identified, accounting for roughly 80% TRR (4.2 ppm) in the surface washes and 0.3% TRR (0.014 ppm) in the organic fraction of the peel extract.

The remaining radioactivity was characterized in the peel (ca. 7% TRR, 0.362 ppm) and pulp (roughly 0.3% TRR, 0.015 ppm) extracts as organic or aqueous-soluble residues based on partitioning with DCM. At least 16 fractions (each present at no more than 0.04 ppm) were isolated from the peel extract organic phase with repetitive TLC and HPLC. The peel extract aqueous phase was further derivatized with pentafluorobenzyl bromide and again partitioned into organic and aqueous fractions; at least six fractions (each at less than 0.05 ppm) were characterized as organo-soluble, and the aqueous phase following derivatization represented 1.21% TRR (0.064 ppm). Radioactivity in the pulp extract was very low; four fractions (each at no more than 0.002 ppm) were characterized as organo-soluble, and the aqueous-soluble residues accounted for 0.01 ppm. Non-extractable residues in the peel were also characterized as organo- or aqueous-soluble following enzyme hydrolysis with pronase; the majority of the enzyme-released radioactivity was aqueous-soluble (3% TRR, 0.157 ppm), and up to five fractions (each at no more than 0.005 ppm) were characterized as organo-soluble residues. None of the isolated organo-soluble residues corresponded to the reference standards used in the study. Based on the results of the orange metabolism study, the petitioner concluded that dithianon is not significantly translocated from the surface of oranges into the peel and pulp. The major residue is unchanged parent; very low levels of dithianon may be converted to numerous complex polar compounds. Based on the results of this study, in conjunction with apple and wheat metabolism studies associated with DP Barcode D312241 (refer to the DERs for MRIDs 44092624 and 44092626), the petitioner concluded that dithianon is not highly metabolized in plants, with a significant amount of the unchanged parent remaining on the plant surface and little to no movement from the application site.

Tabular Summary of Orange Metabolism Study

| **APPENDIX TABLE 3.2.2** **Summary of Characterization and Identification of Radioactive Residues in Oranges Following [$^{14}$C]-Dithianon Treatment.** | | |
|---|---|---|
| **Compound** | **Whole Fruit (28-day PHI)** | |
| | **TRR = 5.26 ppm** | |
| | **% TRR** | **ppm** |
| Dithianon | ~80.3 | 4.2 |
| Organic Extract (Peel and Pulp) [1] | 4.2 | 0.223 |
| Aqueous Extract (Pulp) | 0.2 | 0.01 |
| Derivatized Organo-Soluble (Peel) | 1.5 | 0.08 |
| Derivatized Aqueous-Soluble (Peel) | 1.2 | 0.064 |
| Enzyme Hydrolysate Organo-Soluble (Peel) | 0.2 | 0.01 |
| Enzyme Hydrolysate Aqueous-Soluble (Peel) | 3 | 0.157 |
| Total Identified | ~80 | 4.2 |
| Total Characterized | 10.3 | 0.544 |
| Total Extractable | 97.6 | 5.14 |
| Unextractable (PES) [2] | 2.5 | 0.128 |
| Accountability [3] | 100 | |

1. Includes dithianon identified by HPLC at 0.3% TRR (0.014 ppm).
2. Residues remaining after exhaustive extractions; includes non-extractable pulp and peel.
3. Because the TRR were calculated by summing radioactivity in the surface wash with extractable and non-extractable radioactivity, accountability was 100%.

Executive Summary of Wheat Metabolism Study

American Cyanamid Company (now BASF Corporation) has submitted a study investigating the metabolism of [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}$C]-dithianon (specific activity 28.49 µCi/mg) in spring wheat. The radiolabeled test substance was formulated as a soluble concentrate (SC) and applied as two spray applications to wheat plants (at Zadoks growth stages

59 and 61) at a rate of 1.34 pounds active ingredient per acre (lb ai/A) per application, for a total seasonal rate of 2.68 lb ai/A. The following raw agricultural commodity (RAC) samples were collected: immature wheat (forage stems and ears) was harvested 0 and 20 days after the last application, and mature wheat (grain, husks, and straw) was harvested at a 35-day pre-harvest interval (PHI). The in-life phase of the study was conducted by Huntingdon Research Centre Limited (in Huntingdon, England), and the analytical phase was conducted by Cyanamid Forschung GmbH (in Schwabenheim, Germany).

Total radioactive residues (TRR), determined by summing extractable and non-extractable radioactivity, were 61.0 and 51.9 ppm in/on immature wheat forage stems and ears, respectively, harvested at the 0-day PHI, with 74.9 and 67.6 ppm in/on forage stems and ears, respectively, harvested at the 20-day PHI. TRR were 68.1, 60.6, and 1.91 ppm in/on mature wheat straw, husks, and grain, respectively, harvested 35 days after the last treatment.

Surface washes with acetonitrile (ACN) removed the majority of the radioactivity from forage stems and ears (roughly 81-87% TRR in 0-day samples; roughly 65-67% TRR in 20-day PHI samples), and from wheat grain, husks, and straw (roughly 51-57% TRR). Subsequent extraction with ACN released roughly 4-16% TRR from each matrix. All solvents used for surface washing and extraction contained 1% hydrochloric acid to prevent degradation of dithianon. Non-extractable residues accounted for no more than 10% TRR in 0-day PHI wheat forage stems and ears, and 18.9-35.7% TRR in 20-day PHI forage and mature wheat matrices. Additional radioactivity (roughly 2-20% TRR) was released with acid hydrolysis, leaving approximately 7-25% TRR as non-extractable. Because TRR were determined by summing extractable and non-extractable radioactivity, accountabilities were essentially 100%. Residues were identified by TLC with confirmatory HPLC analysis. Although significant amounts of radioactivity remained non-extractable, these methods, in conjunction with additional enzyme hydrolysis and cell wall component characterization (discussed below), successfully identified the predominant residues in wheat. Adequate storage stability data for wheat straw and grain are available to support the storage conditions of the wheat metabolism samples for the duration of the study (approximately 22.5 months).

Roughly 44-64% TRR were identified in the surface washes/extracts of wheat forage stems, and ears, as well as wheat grain, husks, and straw. Dithianon was the only residue identified, accounting for 58.0-63.6% TRR (38.8-43.4 ppm) in forage stems, 45.3-63.8% TRR (23.5-43.1 ppm) in forage ears, 43.9% TRR (0.84 ppm) in grain, 46.2% TRR (28.0 ppm) in husks, and 53.2% TRR (36.2 ppm) in straw. It was noted that residues of dithianon appeared to decrease in forage stems and increase in forage ears (seedheads) with the longer PHI.

The remaining extractable radioactivity was characterized in wheat matrices as organo- or aqueous-soluble residues based on partitioning with hexane, dichloromethane (DCM), and ethyl acetate. The majority of the radioactivity was determined to be organo-soluble and was comprised of numerous fractions (20 to 25 fractions, each present at less than 10% TRR). None of the organo-soluble fractions corresponded to any of the reference standards used in the study. Aqueous-soluble residues accounted for less than 3% TRR in all matrices.

Non-extractable residues released by acid hydrolysis in all wheat matrices were also characterized as organo- or aqueous-soluble; in general the majority of the acid-released radioactivity was aqueous-soluble, except for in forage stems (20-day PHI) and grain. The organo-soluble residues of the acid hydrolysates of mature wheat matrices were comprised of a large number of unidentifiable peaks. Separate subsamples of wheat straw non-extractable residues were also subjected to base or pronase, cellulase, or pectinase enzyme hydrolysis; base hydrolysis released the largest amount of radioactivity (roughly 12% TRR), and the solubilized radioactivity was characterized as mostly aqueous-soluble in all of the hydrolysates.

The incorporation of radioactivity in the non-extractable residues of wheat straw and grain into various plant components was investigated by sequential extraction/hydrolysis with disodium ethylene-diamine-tetra acetic acid ($Na_2EDTA$) for pectin, dimethylsulfoxide (DMSO) for lignin, and ammonium hydroxide for non-cellulosic polysaccharides or Schweizer's reagent for cellulose. Using this system, in grain and straw, respectively, 14.6% and 2.4% TRR were incorporated into pectin, 15.5% and 10.8% TRR were incorporated into lignin, 2.2% and 4.6% TRR were incorporated into non-cellulosic polysaccharides, and 3.2% and 3.1% TRR were incorporated into cellulose. Non-extractable residues remaining after the cell wall extractions accounted for 2.2-2.4% TRR in grain and 12.0-13.6% TRR in straw. TLC analysis of the straw extracts revealed the presence of several polar compounds primarily retained at the origin.

Based on the results of the wheat metabolism study, the petitioner concluded that dithianon is metabolized to a large number of polar components, none of which are significant. Because of the different functional groups of dithianon, a large range of chemical and enzymatic attacks may occur at various sites of the molecule. The products that are formed may then be further metabolized to natural products such as pectin, lignin, non-cellulosic polysaccharides and cellulose. Based on the results of this study, in conjunction with apple and orange metabolism studies associated with DP Barcode D312241 (refer to the DERs for MRIDs #44092624 and #44092625), the petitioner concluded that dithianon is not highly metabolized in plants, with a significant amount of the unchanged parent remaining on the plant surface and little to no movement from the application site.

<u>Tabular Summary of Wheat Metabolism Study</u>

**APPENDIX TABLE 3.2.3 Summary of Characterization and Identification of Radioactive Residues in Wheat Forage Following [$^{14}$C]-Dithianon Treatment.**

| Compound | 0-day PHI Forage | | | | 20-day PHI Forage | | | |
|---|---|---|---|---|---|---|---|---|
| | Stems | | Ears | | Stems | | Ears | |
| | TRR = 61.0 ppm | | TRR = 51.9 ppm | | TRR = 74.9 ppm | | TRR = 67.6 ppm | |
| | % TRR | ppm | % TRR | ppm | % TRR | ppm | % TRR | ppm |
| Dithianon | 63.6 | 38.8 | 45.3 | 23.5 | 58 | 43.4 | 63.8 | 43.1 |
| Organo-Soluble Unknowns | 26.8 | 15.5 | 46.1 | 23.9 | 14.9 | 11.1 | 16.9 | 11.3 |
| Aqueous-Soluble | 0.7 | 0.41 | 0.5 | 0.27 | 0.7 | 0.5 | 0.6 | 0.39 |
| Acid Hydrolysate | 1.8 | 1.1 | 1.7 | 0.9 | 10.9 | 8.2 | 5.1 | 3.54 |
| Total Identified | 63.6 | 38.8 | 45.3 | 23.5 | 58 | 43.4 | 63.8 | 43.1 |
| Total Characterized | 28.3 | 17 | 48.3 | 25 | 26.5 | 19.8 | 22.6 | 15.1 |
| Total Extractable | 91.6 | 55.8 | 93.5 | 48.6 | 84.3 | 63.2 | 86.2 | 58.3 |
| Unextractable (PES) [1] | 8.4 | 5.1 | 6.5 | 3.4 | 15.7 | 11.7 | 13.8 | 9.3 |
| Accountability [2] | 100 | | 100 | | 100 | | 100 | |

1. Residues remaining after exhaustive extractions; non-extractable residues remaining after acid hydrolysis were not reported but were estimated by the study reviewer.
2. Because the TRR were calculated by summing radioactivity in the surface wash and extractable and non-extractable radioactivity, accountability was 100%.

| APPENDIX TABLE 3.2.4 | Summary of Characterization and Identification of Radioactive Residues in Mature Wheat Matrices Following [$^{14}$C]-Dithianon Treatment. | | | | | |
|---|---|---|---|---|---|---|
| Compound | Grain | | Husks | | Straw | |
| | TRR = 1.91 ppm | | TRR = 60.6 ppm | | TRR = 68.1 ppm | |
| | % TRR | ppm | % TRR | ppm | % TRR | ppm |
| Dithianon | 43.9 | 0.84 | 46.2 | 28 | 53.2 | 36.2 |
| Organo-Soluble Unknowns | 18.3 | 0.36 | 19.4 | 11.7 | 15.6 | 10.6 |
| Aqueous-Soluble | 2.3 | 0.04 | 1.4 | 0.88 | 1.3 | 0.88 |
| Acid Hydrolysate | 20.3 | 0.4 | 7.9 | 4.8 | NC [1] | NC |
| Base Hydrolysate | NC | NC | NC | NC | 11.7 | 7.9 |
| Total Identified | 43.9 | 0.84 | 46.2 | 28 | 53.2 | 36.2 |
| Total Characterized | 40.9 | 0.8 | 28.7 | 17.4 | 28.6 | 19.4 |
| Total Extractable | 84.7 | 1.63 | 74.9 | 45.4 | 81.8 | 55.7 |
| Unextractable (PES) [2] | 15.4 | 0.3 | 25.1 | 15.2 | 18.2 | 12.5 |
| Accountability [3] | 100 | | 100 | | 100 | |

1. NC = Not Conducted (extraction step and/or characterization analysis was not conducted for matrix in question).
2. Residues remaining after exhaustive extractions; non-extractable residues remaining after acid hydrolysis were not reported but were estimated by the study reviewer.
3. Because the TRR were calculated by summing radioactivity in the surface wash and extractable and non-extractable radioactivity, accountability was 100%.

## A-3.3 NATURE OF THE RESIDUE - RUMINANTS

**DER for MRID #44092627 (Goat Metabolism Studies)**

**Ruminants:** American Cyanamid submitted the results of two studies (conducted in 1989 to 1990, and 1992 to 1994) investigating the metabolism of [$^{14}$C]-dithianon in lactating goats. In the first study (**Study 1**), [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}$C]-dithianon (with specific activity of 58.6 mCi/mmole) was administered orally to two lactating goats at 3 ppm (low dose) and 30 ppm (high dose) in the diet. The dosing levels represent 1X and 10X the maximum theoretical dietary burden (MTDB) for dairy cattle, and 0.5X and 5X the MTDB for beef cattle (see Table 5). In the second study (**Study 2**), [$C_5$,$C_6$,$C_9$,$C_{10}$-naphthoquinone-$^{14}$C]-dithianon (with specific activity of 21.57 µCi/mg) was administered orally to a lactating goat at 25 ppm in the diet. The dosing level represents 4.2X the MTDB for beef cattle, and 8.3X the MTDB for dairy cattle. In both studies, the goats were dosed once per day for 5 consecutive days. Milk was collected twice daily, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice (5 to 6 hours after the last dose). The in-life and analytical phases of **Study 1** were conducted by Hazleton Laboratories Incorporated (in Madison, WI). For **Study 2**, the in-life phase was conducted by Inveresk Research International Limited (in Tranent, Scotland), and the analytical phase was conducted by Sittingbourne Research Centre (in Sittingbourne, England).

In **Study 1**, TRR in samples from the low dose and high dose goat, respectively, were:

(A) non-detectable (ND) to 0.003 ppm, and 0.018 to 0.030 ppm in milk,
(B) 0.019 and 0.174 ppm in liver,
(C) 0.065 and 0.489 ppm in kidney,
(D) ND and 0.013 ppm in muscle,
(E) ND and 0.014 ppm in omental fat, and
(F) 0.003 and 0.013 ppm in renal fat.

Radioactivity was highest in liver and kidney and lowest in muscle and fat. Residues in milk appeared to peak in milk collected 32 hours after the first dose and plateaued thereafter. A large portion of the administered dose was excreted, with urine and feces together accounting for roughly 78% of the administered dose in low and high dose goats.

In **Study 2**, TRR were 0.003 to 0.016 ppm in milk, 0.157 ppm in liver, 0.475 ppm in kidney, 0.012 and 0.014 ppm in fore and hind leg muscle, and 0.074 and 0.009 ppm in subcutaneous and renal fat, respectively. Radioactivity was highest in liver and kidney and lowest in milk, muscle, and renal fat. Residues in milk appeared to peak in milk collected 48 hours after the first dose and plateaued thereafter. A large portion of the administered dose was excreted, with urine and feces together accounting for roughly 66% of the administered dose, while 27.3% TRR were recovered from the gastrointestinal tract.

Similar extraction methods based on the Bligh-Dyer procedure were used in the two studies. In **Study 1**, samples of milk, liver, kidney, and muscle from the high dose goat were extracted with methanol/chloroform. This procedure extracted the majority of the radioactivity (roughly 77%) in milk, but was less successful in liver, kidney, and muscle, in which roughly 49%, 52%, and 32% TRR were extracted. Of the extractable residues, roughly 24 to 48% of the TRR in milk, liver, kidney and muscle were aqueous-soluble (aqueous methanol extract) while roughly 6 to 29% TRR were organo-soluble (chloroform extract). Non-extractable residues accounted for 20.2% TRR (0.005 ppm) in milk, 54.7% TRR (0.095 ppm) in liver, 73.6% TRR (0.360 ppm) in kidney, and 95.2% TRR (0.012 ppm) in muscle; the petitioner made no attempts to release non-extractable residues in liver, kidney, and muscle. Residues in the aqueous methanol and chloroform extracts were characterized primarily by HPLC analysis. Adequate storage stability data are available to support this study.

In **Study 2**, samples of liver, kidney, and muscle were extracted with methanol/chloroform, and fat was extracted with DCM. Milk fractions (cream, curd, and whey) were extracted with methanol, but only for purposes of determining the distribution of TRR in the individual fractions. The extraction procedures released roughly 41% TRR in kidney, and roughly 20 to 27% TRR in liver, muscle, and fat; methanol extraction of milk released roughly 78% TRR. Of the extractable residues, roughly 18% and 36% of the TRR in liver and kidney were aqueous-soluble (aqueous methanol extract) while roughly 8% and 4% TRR were organo-soluble (chloroform extract); however, in muscle the distribution was nearly even, with 9.6% TRR aqueous-soluble and 11% TRR organo-soluble. Non-extractable residues accounted for 21.7% TRR (0.003 ppm) in milk, 72.7% TRR (0.114 ppm) in liver, 59.4% TRR (0.282 ppm) in kidney, 80.0% TRR (0.010 ppm) in muscle, and 73.9% TRR (0.055 ppm) in fat. The non-extractable residues in liver were subjected to a number of enzyme and acid/base hydrolysis procedures, which were successful in releasing up to 73% TRR (0.115 ppm, 0.05 M NaOH hydrolysis), and non-extractable residues in kidney were subjected to base hydrolysis followed by enzyme hydrolysis, which released 41.5% TRR (0.197 ppm). Non-extractable residues in liver and kidney following various hydrolysis procedures ranged 3.9% to 75.8% TRR (0.006 to 0.119) ppm in liver, and accounted for 13.0% TRR (0.062 ppm) in kidney. Residues were characterized by HPLC and/or TLC analysis. Adequate storage stability data are available to support this study.

In **Study 1**, dithianon accounted for 8.2% TRR in milk, 1.0% TRR in liver, and 2.3% TRR in kidney; no further residues were identified. Up to six discrete unknowns (designated A-1 through A-6) were determined in the aqueous methanol extracts of milk, liver and kidney, while four discrete unknowns (designated B-1 through B-4) were determined in the ACN phases of the chloroform extracts of milk and liver following partitioning with ACN and hexane; the ACN phase of kidney was not analyzed. Individual unknowns in the methanol extracts were present at up to 10.4% TRR (0.003 ppm) in milk, 12.1% TRR (0.021 ppm) in liver, and 6.74% TRR (0.033 ppm) in kidney. Individual unknowns in the ACN phases were present at up to

4.47% TRR (0.001 ppm) in milk and 2.06% TRR (0.004 ppm) in liver. None of the metabolites corresponded to the single reference standard for the dicarboxylic acid anhydride of dithianon that was used in the study. Approximately 6 to 7% TRR in milk and liver and 2% TRR in kidney partitioned into hexane suggesting incorporation of radioactivity into lipids.

Although no further characterization/identification was attempted in milk and tissues in **Study 1**, the petitioner subjected urine to further procedures to characterize unknowns observed in milk and tissues. Subsamples of urine were subjected to enzymatic hydrolysis with aryl sulfatase and β-glucuronidase for characterization of residues. Selected components in urine were also isolated by solid-phase extraction (SPE) and/or semi-preparative HPLC. Enzyme hydrolysis suggested the presence of glucuronide conjugates of the parent (tentatively proposed to be Unknowns A-2 and A-3 by the petitioner), and possibly other metabolites, and indicated that sulfate conjugates did not form. Infrared spectrometry of Unknown A-3 isolated from urine suggested the loss of a nitrile functional group.

In **Study 2** only liver and kidney extracts were subjected to HPLC and TLC analyses. No qualitative or quantitative results were reported; however, the chromatograms that were submitted confirmed a complex metabolite profile. The petitioner indicated that semi-preparative HPLC was somewhat successful in resolving the complex pattern of metabolites in the organic and aqueous phases of liver and kidney, but that no system was able to resolve all of the components. The petitioner was able to conclude merely that metabolites exhibiting a wide range of polarity were present. Co-chromatography attempts appeared to be limited to the final aqueous phase of kidney and the organic and/or aqueous phases following hydrolysis procedures in liver and kidney; remaining identification was attempted by retention time comparisons. Co-chromatography of the final aqueous phase of kidney with a mixture of the reference standards indicated that only phthalic acid was a potential metabolite in the extract. TLC analysis of the chloroform and methanol/water phases of liver and kidney were less successful. The hexane phases were not analyzed because they contained large amounts of co-extractive material, and the attempted HPLC of the chloroform phases was unsuccessful due to column blocking. The petitioner stated that comparison of the HPLC chromatograms of the organic and aqueous phases of liver and kidney indicated that there were some similarities in the metabolite profiles, but that because the profiles were very complex, comparisons were difficult. The petitioner stated that no single metabolite accounted for greater than 0.05 ppm, and stated that no parent (dithianon) was present in the extracts; the petitioner also stated that none of the metabolites represented by the reference standards were identified.

Additional non-extractable radioactivity in liver was solubilized by treatment with pepsin, which released 18% TRR, or NaOH, which released up to 73% TRR; hydrolysis with glucuronidase and sulfatase released no more than 1% TRR, and acid hydrolysis with 1 M and 6 M HCl released only 6 to 7% TRR. Results of hydrolysis with pancreatin were equivocal because they did not differ from results for control samples run with buffer solution; therefore, the petitioner concluded that residues (9.0% TRR) were released by the buffer solution. In kidney, hydrolysis with NaOH followed by pancreatin released up to 58% TRR.

Based on pepsin hydrolysis results, the petitioner concluded that non-extractable residues in liver were largely bound to proteins, which would partially account for the high levels of non-extractable residues. Although the results of HPLC and TLC analysis of the various hydrolysates and the corresponding organic and aqueous phases following partitioning were variable, some useful results were obtained. TLC analysis of the aqueous phase of the 6 M NaOH hydrolysate of liver following partitioning with diethyl ether was successful in demonstrating the absence of five of the reference standard metabolites. Sequential filtration of the aqueous phase through 50K, 10K, 1000, and 500 molecular weight (mw) cut-off membranes indicated that the radioactivity was associated with small molecules (less than 500 mw), and HPLC analysis of the aqueous phase and corresponding organic phase (and retention time comparisons with the reference standards) suggested that only phthalic acid was a potential metabolite in these phases. Similar comparisons of the organic phase of the 6 M NaOH hydrolysate obtained following β-glucuronidase and sulfatase hydrolyses via TLC confirmed the absence of dithianon and a pyrrolidine dione metabolite, and HPLC analysis of the corresponding organic and aqueous phases indicated that only phthalic acid was a potential metabolite. TLC analysis of the organic phase following pancreatin hydrolysis was successful in resolving at least seven components, and HPLC analysis of the corresponding aqueous phase suggested that phthalic acid was again the only potential metabolite. The petitioner noted that co-chromatography of isolated residues was not possible due to the low levels of radioactivity in the organic and aqueous phases, and complexity of the profiles.

TLC analysis of the organic and aqueous phases of kidney following 6 N NaOH hydrolysis and pancreatin hydrolysis of the non-extractable residues was unsuccessful, with the majority of radioactivity eluting with the solvent front. HPLC co-chromatography of the same organic and aqueous phases with mixed reference standards indicated that only phthalic acid was a potential metabolite in any of the phases.

The petitioner also conducted *in vitro* experiments with glutathione and N-acetyl-cysteine, which indicated that dithianon reacted very quickly with glutathione and protein thio groups, even in the absence of enzymes such as glutathione transferase. The results of this study suggested that conjugation was a likely pathway of metabolism in goats.

Based on the results of the goat metabolism studies, the petitioner concluded that dithianon is extensively metabolized in ruminants, with the most likely first step being the opening of the dithiine ring by protein thiols. Following initial ring opening, the molecule would undergo a number of further bio-transformations yielding metabolites with chemical structures very different from the original molecule. This conclusion is supported by the finding of phthalic acid as the only identifiable potential metabolite in ruminant tissues.

***Conclusions:*** The submitted goat metabolism study is adequate to satisfy data requirements. Although the extraction and chromatographic procedures yielded only limited qualitative and quantitative data, the analytical results for milk and tissues, in conjunction with the urinalysis results and the *in vitro* enzyme studies, confirmed that dithianon is extensively metabolized in goats. The study results are consistent with what would be expected based on the molecular structure, which is highly susceptible to attack at three reactive sites: the C=O bonds, the C-S-C bonds, and the cyano bond. The study results are also consistent with the results of the rat metabolism study (refer to the Tox DER for MRID #44092622), which demonstrated that dithianon was extensively degraded. HED concludes that the ROC in ruminant commodities is dithianon *per se*.

**Poultry:** There are no significant poultry feed items associated with the proposed uses of dithianon. Therefore, poultry metabolism data are not required to support the current petition.

## A-4.0 CONFINED ROTATIONAL CROP STUDIES

As all proposed uses in this tolerance petition are on imported commodities, confined rotational crop studies are not relevant to this assessment.

## A-5.0 ANALYTICAL METHODOLOGY

| APPENDIX TABLE 5.1 Summary of Parameters for the Proposed Tolerance-Enforcement Method Used for the Quantitation of Dithianon Residues in Pome Fruit. | |
|---|---|
| Method ID | HUK 460/38-01R |
| Analyte | Dithianon |
| Extraction Solvent/Technique | Samples are extracted with acidified ACN (twice), and the resulting extracts are combined and partitioned with hexane (twice). |
| Cleanup Strategies | If necessary, the acidified hexane phase is applied to a silica gel column, and residues are eluted with 1% acetic acid in DCM. |
| Instrument/Detector | HPLC utilizing a silica column and an isocratic mobile phase of ACN/water/glacial acetic acid/tetrahydrofuran (700:300:1:10 vol/vol/vol/vol), with UV detection (254 nm). |
| Standardization Method | External standardization using a calibration curve prepared by injecting constant volumes of calibration standard solutions. The calibration curve is calculated by linear regression (least squares fit) using peak response. |
| Stability of Standard Solutions | Not addressed. |
| Retention Times | Approximately 6 minutes. |

| APPENDIX TABLE 5.2 Summary of Parameters for the Proposed Tolerance-Enforcement Method Used for the Quantitation of Dithianon Residues in Hops. | |
|---|---|
| Method ID | M 2600 |
| Analyte | Dithianon |
| Extraction Solvent/Technique | Samples are extracted with ACN/HCl (2:1 vol/vol), and the resulting extract is partitioned with DCM. |
| Cleanup Strategies | The DCM phase is purified by GPC, and residues are eluted with 1% acetic acid in methanol. |
| Instrument/Detector | HPLC utilizing a silica column and an isocratic mobile phase of ACN/0.3 M ammonium acetate at pH 4.5 (65:35 vol/vol), with electro-conductivity detection. |
| Standardization Method | External standardization using a calibration curve prepared by injecting constant volumes of calibration standard solutions over a concentration range of 0.25 to 2 times the working standard. The response factor is calculated from the peak responses of the standards. |
| Stability of Standard Solutions | Not addressed, except that it was noted that the stock solution was to be prepared monthly and stored refrigerated in amber bottles. |
| Retention Time | Roughly 3.8 minutes. |

## A-6.1 SUMMARY OF MAGNITUDE OF THE RESIDUE STUDIES FOR POME FRUIT, CROP GROUP 11)

Crop field trials are conducted to determine the maximum residue which may be expected in/on a raw agricultural commodity as a result of the legal use of the pesticide. The trials must reflect label directions which would be expected to result in the maximum residue levels; ergo, the trials should employ maximum label rates, maximum number of applications, minimum re-treatment interval(s), and minimum pre-harvest interval.

Data generated in the U.S. or countries other than those where the petitioner has existing or proposed uses may be substituted for up to half of the required number of foreign trials, but a minimum of three trials must be from the countries in which the pesticide is marketed. The petitioner should demonstrate that crop cultural practices, climatological conditions, and use patterns are substantially similar between the subject foreign regions and regions represented by the U.S. (or other) data.

| APPENDIX TABLE 6.1 Summary of Residues from the Pome Fruit and Hops Field Trials with Dithianon. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Country | Single Application Rate (lb ai/A) [kg ai/ha] | Total Application Rate (lb ai/A) [kg ai/ha] | PHI (Days) | Residue Levels (ppm) | | | | | |
| | | | | n | Min. | Max. | HAFT [1] | Mean | Std. Dev. |
| **Apple** | | | | | | | | | |
| Registered use in France. Multiple foliar broadcast applications of the 750 g/L SC formulation at 0.335 lb ai/A per application (0.375 kg ai/ha per application), with a 28-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| France | 0.468 [0.525] | 7.96 [8.92] | 14 | 2 | <0.05 | 1.36 | 0.70 | 0.70 | 0.94 |
| | 0.468 [0.525] | 6.56 [7.35] | 21 | 1 | 1.73 | 1.73 | 1.73 | 1.73 | NA [2] |
| | 0.468 [0.525] | 6.09 [6.82] | 84 | 1 | 0.70 | 0.70 | 0.70 | 0.70 | NA |
| | 0.937 [1.05] | 12.2 [13.6] | 84 | 1 | 1.36 | 1.36 | 1.36 | 1.36 | NA |

| APPENDIX TABLE 6.1 Summary of Residues from the Pome Fruit and Hops Field Trials with Dithianon. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Country | Single Application Rate (lb ai/A) [kg ai/ha] | Total Application Rate (lb ai/A) [kg ai/ha] | PHI (Days) | Residue Levels (ppm) | | | | | |
| | | | | n | Min. | Max. | HAFT [1] | Mean | Std. Dev. |
| Registered use in New Zealand. Multiple foliar broadcast applications of the 500 g/L SC formulation at 0.22 lb ai/A per application (0.25 kg ai/ha per application), with a 42-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| New Zealand | 0.669-0.836 [0.750-0.938] | 7.53 [8.44] | 16 | 2 | 0.21 | 0.24 | 0.24 | 0.23 | 0.02 |
| | 0.669 [0.750] | 6.02 [6.75] | 21 | 2 | 1.26 | 2.47 | 1.87 | 1.87 | 0.86 |
| | 1.07-1.34 [1.20-1.50] | 12.0 [13.5] | 16 | 1 | 0.19 | 0.19 | 0.19 | 0.19 | NA |
| Registered use in Germany. A maximum of 12 foliar broadcast applications of the 750 g/L SC formulation at 0.67 lb ai/A per application (0.75 kg ai/ha per application), with a 21-day PHI. A maximum seasonal rate of 8.03 lb ai/A (9.00 kg ai/ha) was calculated by the study reviewer. | | | | | | | | | |
| Germany | 0.451-0.494 [0.505-0.554] | 5.63-5.74 [6.32-6.43] | 21 | 4 | 0.36 | 0.76 | 0.76 | 0.56 | 0.17 |
| Registered use in Brazil. Multiple foliar broadcast applications of the 750 g/kg WP formulation at 1.673 lb ai/A per application (1.875 kg ai/ha per application), with a 21-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| Brazil | 0.075% | 0.075% | 21 | 4 | 0.24 | 0.89 | 0.89 | 0.50 | 0.28 |
| | | | 35 | 4 | 0.14 | 0.54 | 0.54 | 0.31 | 0.17 |
| | 0.125% | 0.125% | 21 | 4 | 0.64 | 0.95 | 0.95 | 0.73 | 0.15 |
| | | | 35 | 4 | 0.26 | 0.50 | 0.50 | 0.34 | 0.11 |
| | 0.250% | 0.250% | 21 | 4 | 0.07 | 0.95 | 0.95 | 0.68 | 0.41 |
| | | | 35 | 4 | 0.04 | 0.80 | 0.80 | 0.45 | 0.32 |
| **Pear** | | | | | | | | | |
| Registered use in Australia. Multiple foliar broadcast applications of the 750 g/L SC and 750 g/kg WP formulations at 1.34 lb ai/A per application (1.50 kg ai/ha per application), with a 21-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| Australia | 0.075% | 0.075% | 21 | 4 | 1.79 | 3.67 | 3.67 | 2.54 | 0.88 |
| | 0.15% | 0.15% | 21 | 2 | 1.16 | 1.98 | 1.98 | 1.57 | 0.58 |
| | NS [3] | NS | 21 | 2 | 1.71 | 3.49 | 3.49 | 2.60 | 1.26 |
| Registered use in New Zealand. Multiple foliar broadcast applications of the 500 g/L SC formulation at 0.31 lb ai/A per application (0.35 kg ai/ha per application), with a 42-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| New Zealand | 0.669 [0.750] | 7.36 [8.25] | 21 | 1 | 0.26 | 0.26 | 0.26 | 0.26 | NA |
| | 1.07 [1.20] | 11.8 [13.2] | 21 | 1 | 0.33 | 0.33 | 0.33 | 0.33 | NA |
| | 0.0005 [4] | 0.0005 [4] | 25 | 1 | 0.14 | 0.14 | 0.14 | 0.14 | NA |
| Registered use in France. Multiple foliar broadcast applications of the 750 g/L SC formulation at 0.335 lb ai/A per application (0.375 kg ai/ha per application), with a 28-day PHI. Maximum seasonal rate was not provided. | | | | | | | | | |
| France [5] | 0.468 [0.525] | 5.62-6.09 [6.30-6.82] | 26-28 | 2 | 0.43 | 0.60 | 0.60 | 0.52 | 0.12 |
| | 0.468 [0.525] | 6.09 [6.82] | 95 | 1 | <0.07 | <0.07 | <0.07 | <0.07 | NA |
| | 0.937 [1.05] | 12.2 [13.6] | 28 | 1 | 1.79 | 1.79 | 1.79 | 1.79 | NA |
| **Hops** | | | | | | | | | |
| Registered use in Germany. A maximum of 10 foliar broadcast applications of the 750 g/L SC formulation at 0.67 lb ai/A per application (0.75 kg ai/ha per application), for a total seasonal rate of 6.69 lb ai/A (7.50 kg ai/ha), with a 21-day PHI. | | | | | | | | | |

| APPENDIX TABLE 6.1 Summary of Residues from the Pome Fruit and Hops Field Trials with Dithianon. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Country** | **Single Application Rate (lb ai/A) [kg ai/ha]** | **Total Application Rate (lb ai/A) [kg ai/ha]** | **PHI (Days)** | **Residue Levels (ppm)** | | | | | |
| | | | | **n** | **Min.** | **Max.** | **HAFT [1]** | **Mean** | **Std. Dev.** |
| Germany [Green Cones] | 0.377-1.34 [0.423-1.50] | 8.45-11.3 [9.47-12.7] | 0 | 22 | 11.3 | 161 | 155 | 46.6 | 40.3 |
| | | | 7-8 | 23 | 6.06 | 143 | 143 | 46.5 | 40.0 |
| | | | 10 | 16 | 10.5 | 92.7 | 91.8 | 38.5 | 24.6 |
| | | | 14-15 | 25 | 4.18 | 127 | 123 | 33.3 | 32.1 |
| | | | 21 | 8 | 2.63 | 13.9 | 13.8 | 7.65 | 4.73 |
| Germany [Dried Cones] | 0.377-1.34 [0.423-1.50] | 8.45-11.3 [9.47-12.7] | 10 | 16 | 70.4 | 200 | 193 | 118 | 51.3 |
| | | | 14-15 | 23 | 13.1 | 243 | 242 | 85.3 | 56.5 |
| | | | 21 | 8 | 8.80 | 59.5 | 58.9 | 29.0 | 20.5 |

1. HAFT = Highest Average Field Trial.
2. NA = Not Applicable.
3. NS = Not Specified.
4. Concentration (units not specified).
5. Residue results for pear trials in France have been corrected for potential decline during storage.

## A-6.2 MAGNITUDE OF THE RESIDUE - LIVESTOCK

The only livestock feed item associated with the proposed uses is apple wet pomace. There are no poultry feed items associated with the proposed uses. HED has evaluated the MTDB for livestock, and concludes that the proposed uses of dithianon in this petition result in a 40 CFR §180.6[a][3] situation for ruminant commodities; specifically, there is no reasonable expectation of finite residues in ruminant commodities.

## A-7.0 INTERNATIONAL CONSIDERATIONS

CODEX MRLs have been established for residues of dithianon on pome fruit at 5 mg/kg, and on hops at 100 mg/kg: the proposed tolerances on imported commodities are harmonized with established MRLs. There are currently no Canadian, Mexican or Codex MRLs for dithianon. The MRL status sheet is attached.

## A-7.0 INTERNATIONAL CONSIDERATIONS (cont.)

| INTERNATIONAL RESIDUE LIMIT STATUS | | | |
|---|---|---|---|
| **Chemical Name:**<br>5,10-dihydro-5,10-dioxonaphtho(2,3-*b*)-1,4-dithiin-2,3-dicarbonitrile | **Common Name:**<br>Dithianon | **X Proposed Tolerances**<br>☐ Reevaluated tolerance<br>☐ Other | **Date:** 8/5/2005 |
| Codex Status (Maximum Residue Limits) | | **US Tolerances (Recommended)** | |
| ☐ No Codex proposal step 6 or above<br>☐ No Codex proposal step 6 or above for the crops requested | | **Petition Number:** 6E4781<br>**DP Barcode:** D312241<br>**Other Identifier:** PC Code 099201 | |
| Residue definition (step 8/CXL): Dithianon | | **Reviewer (Branch):** William T. Drew (RAB2) | |
| | | **Residue Definition:** Dithianon | |
| Crops | MRL (mg/kg) | **Crops** | **Tolerance (ppm)** |
| Pome Fruit | 5 | Pome Fruit (Crop Group 11) | 5 |
| Hops | 100 | Dried Hops Cones | 100 |
| | | | |
| | | | |
| | | | |
| | | | |
| Limits for Canada | | Limits for Mexico | |
| X No Limits<br>☐ No Limits for the crops requested | | X No Limits<br>☐ No Limits for the crops requested | |
| Residue definition: NA | | Residue definition: NA | |
| Crop(s) | MRL (mg/kg) | Crop(s) | MRL (mg/kg) |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| **NOTES:** per Stephen Funk, 8/5/2005. NA = Not Applicable. | | | |

## A-8.0 ENVIRONMENTAL CONSIDERATIONS

As all proposed uses in this tolerance petition are on imported commodities, environmental degradation is not relevant to this assessment.